

# Epitope 9

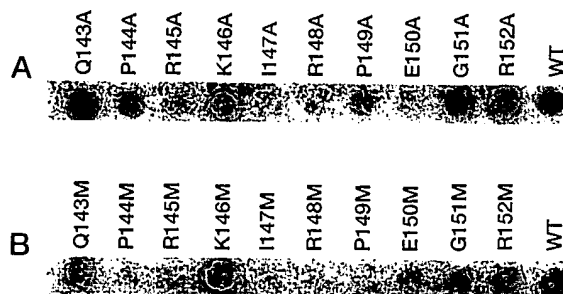


FIG. 1. Single amino acid changes to epitope 9 result in loss of IgE binding to this epitope. Epitope 9 was synthesized with an alanine (Panel A) or methionine (Panel B) residue substituted for one of the amino acids and probed with a pool of serum IgE from 15 patients with documented peanut hypersensitivity. The letters across the top of each panel indicate the one-letter amino acid code for the residue normally at the position and the amino acid that was substituted for this residue. The numbers indicate the position of each residue in the Ara h1 protein. WT, indicates the wild type peptide (no amino acid substitutions).

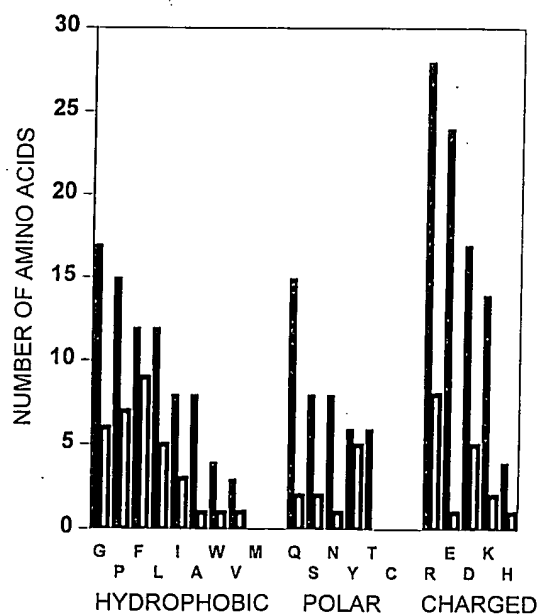


FIG. 2. Hydrophobic amino acids are more critical to IgE binding. The type of each amino acid within the Ara h1 epitopes was assessed relative to its importance to IgE binding. The closed boxes represent the total number of a particular type of amino acid residue found in all of the Ara h1 epitopes, whereas the open boxes represent the number of that type of residue which, when replaced, was found to result in the loss of IgE binding.

# A

NNPFYFPSRR FSTRYGNQNGRIRVLQRFQDQSRQFQNLQNHRIVQIEAKPNTLVLP 227  
DNPFYFNSDNSWNTLFKNQYGHIRVLQRFQDQSKRLQNLQLEDYRLVEFRSKPETLLLL

KHADADNILVIQQQATVTVANGN NRKSFNLDEGH ALRIPSGFISYILNRH 278  
QQADAELLVVRSGSAILVLVKPDDRREYFFLTSDNPISDHQKIPAGTIFYLVNPD

DNQNLRVAKISMPVNTPGQFEDFFPASSRDQSSYLQGFSRNTLEAAFNAEFNEIRRV 335  
PKEDLRITQLAMPVNNPQIH EFFLSSTEAAQSSYLQEFKSKHILEASFNSKFEEINRV

LLEENAGGEQEERGQRRWSTRSSENNEGVIKVSKEHVEELTKHAKSVSKKGSEEE 391  
LFEEEGQQEGV IVNIDSEKIKELSKHAKSSSRKSLSKQD

GDITNPINLREGEPLDLSNNFGKLFVVKPKDKNPQLQDLDMMLTCVEIKEGALMLPHF 448  
NTIGNEF GNLTERTDNSLN VLISSIEEAGALFVPHY

NSKAMVIVVVNKGKTGNLELVAVRKEQQQRGRREEEEDDEEEEGSNREVRRTARLK 505  
YSKAIVILVVNEGEAHVELVGPKNKETLEYE SYRAELS

EGDVFIIMPAHPVAINASSELHLLGFGINAENNRIFLAGDKDNVIDQIE KQ 557  
KDDVFIIPAAYPVAIKATSNVNFTGFGINANNNRNLLAGKTDNVISSIGRALDGKD

AKDLAFPGSGEQVEKLIKQKESHFVSAR 586  
VLGLTFSGSGDEVKLINKQSGSYFVDAH

# B

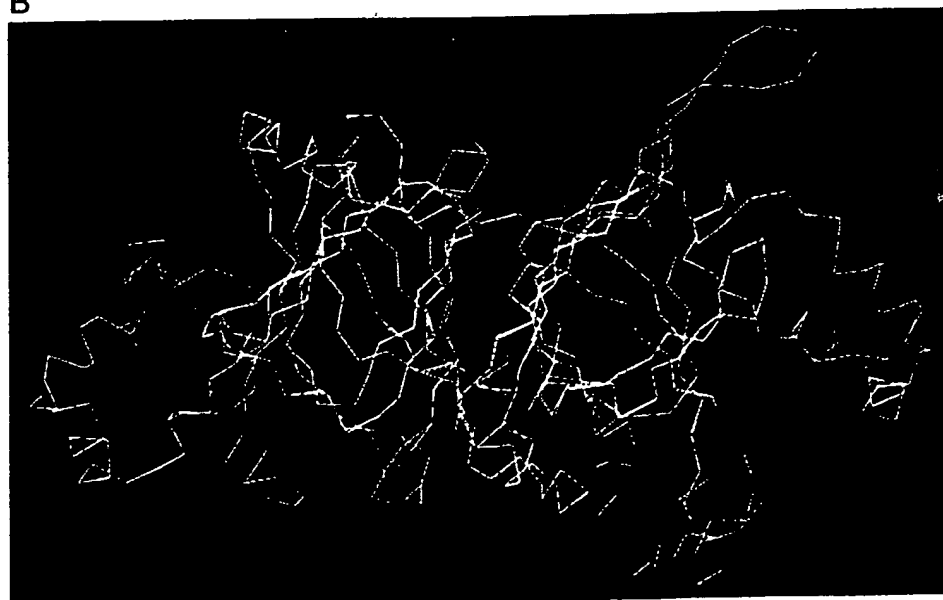


FIG. 3. Alignment of the primary amino acid sequences and the  $\alpha$ -carbon structural alignment of Ara h1 and the phaseolin A chain. Panel A represents the single letter amino acid code for Ara h1 residues 172-586 (*top line*) and all of the phaseolin A chain (*bottom line*). The structurally conserved regions, shown in **bold type**, were used to develop the initial backbone of the Ara h1 model. The other regions were used in protein loop searches to complete the tertiary structure of Ara h1. Panel B represents the  $\alpha$ -carbon alignment of the final model of Ara h1 (*white*) versus the phaseolin A chain (*yellow*). Labeled residues Asn<sup>1</sup> and Arg<sup>415</sup> represent the N and C termini of the Ara h1 model, respectively. Areas between labeled amino acids Asn<sup>169</sup>-Val<sup>193</sup>, Val<sup>212</sup>-Gly<sup>221</sup>, Phe<sup>240</sup>, Pro<sup>226</sup>, Pro<sup>226</sup>-Phe<sup>240</sup> and Arg<sup>300</sup>-Asn<sup>323</sup> represent areas of structural uncertainty due to insertions in Ara h1 or unsolved sequences in phaseolin. Note that the residue numbers are shifted due to the N-terminal deletion from the Ara h1 coding sequence found in the GenBank™ data bank (the amino acid sequence of this protein can be accessed through the GenBank™ data bank under GenBank Accession Number L34402 (17)).

# Phi/Psi Plot

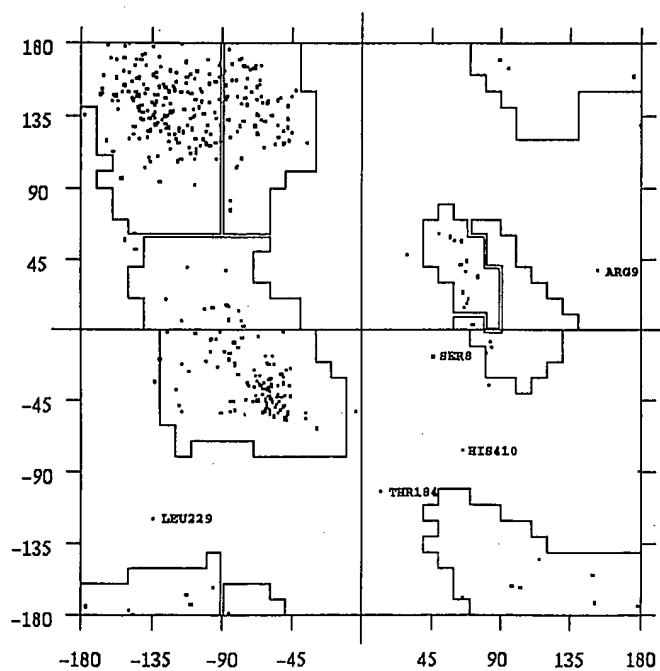


FIG. 4. Most of the  $\phi/\psi$  torsion angles of the amino acid residues in the Ara h1 tertiary structure model are allowed. A plot of the  $\phi$  and  $\psi$  angles for the amino acids in the Ara h1 tertiary structure model is shown. Each dot within one of the boxes represents an amino acid that has acceptable torsion angles. Major outliers are indicated by their three letter amino acid code and position using the N-terminal as residue 1 as in Fig. 3.

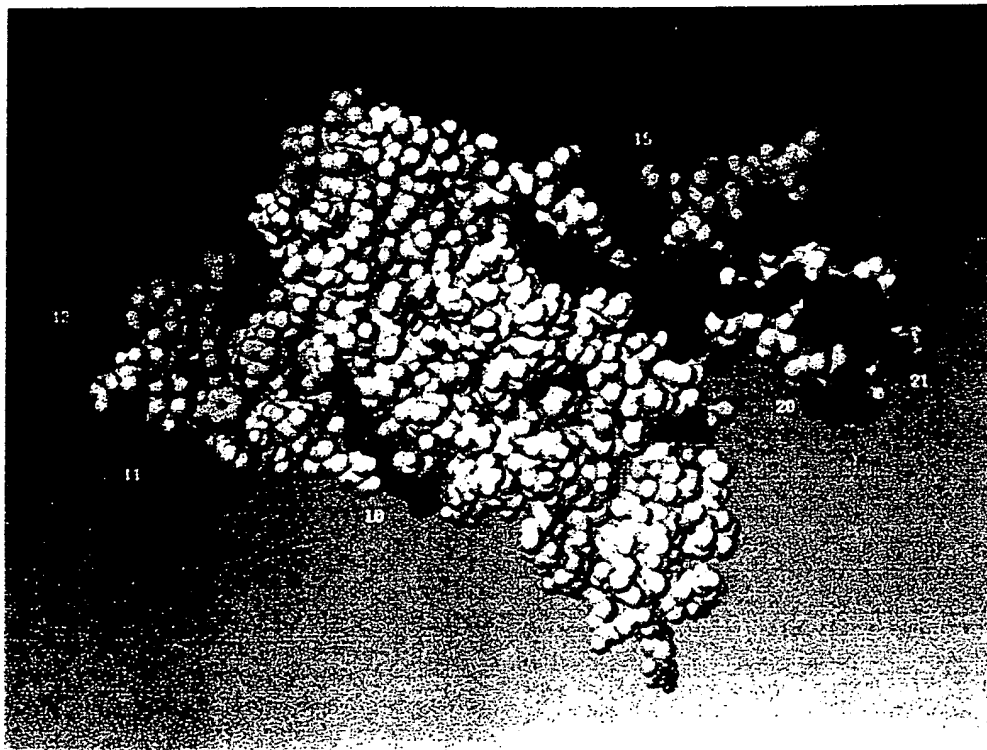
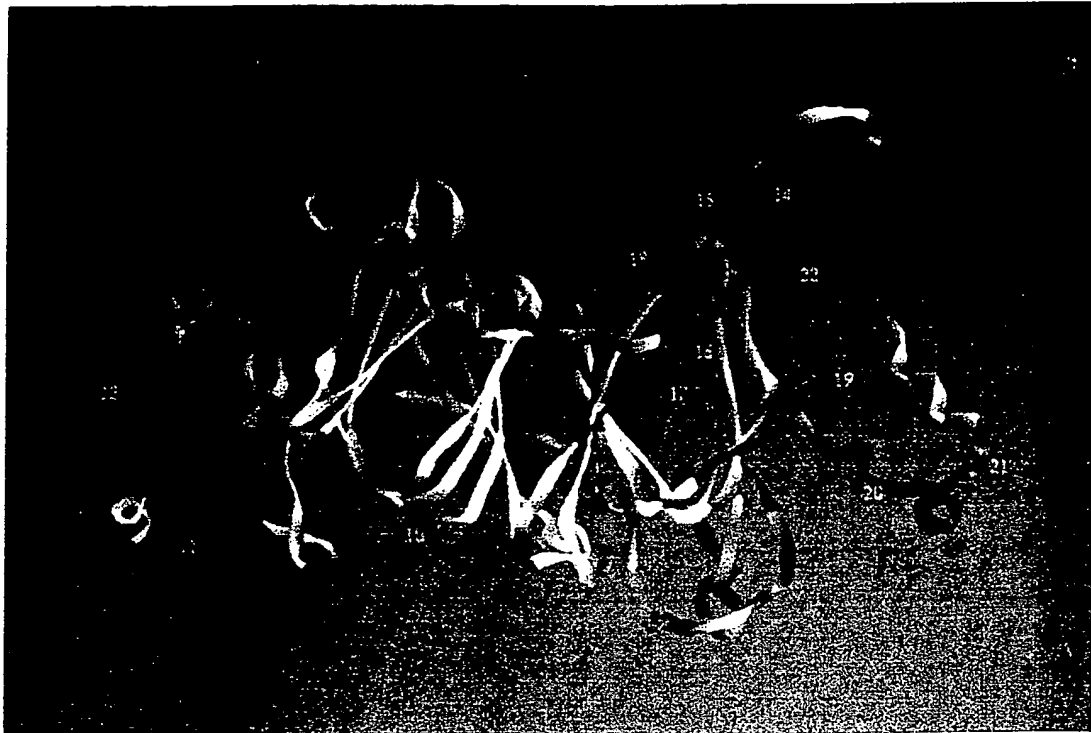


FIG. 5. The majority of the Ara h1 IgE binding epitopes are clustered in two regions of the allergen. The *top panel* represents a ribbon diagram of Ara h1 tertiary structure. The *numbered red areas* are IgE binding epitopes 10–22. Epitopes 13 and portions of 14 and 15 lie in an area of structural uncertainty. The *bottom panel* is a space filling model of Ara h1 tertiary structure. The *red areas* represent the IgE binding epitopes, and the *yellow atoms* are the residues that were determined to be critical for IgE binding to occur.

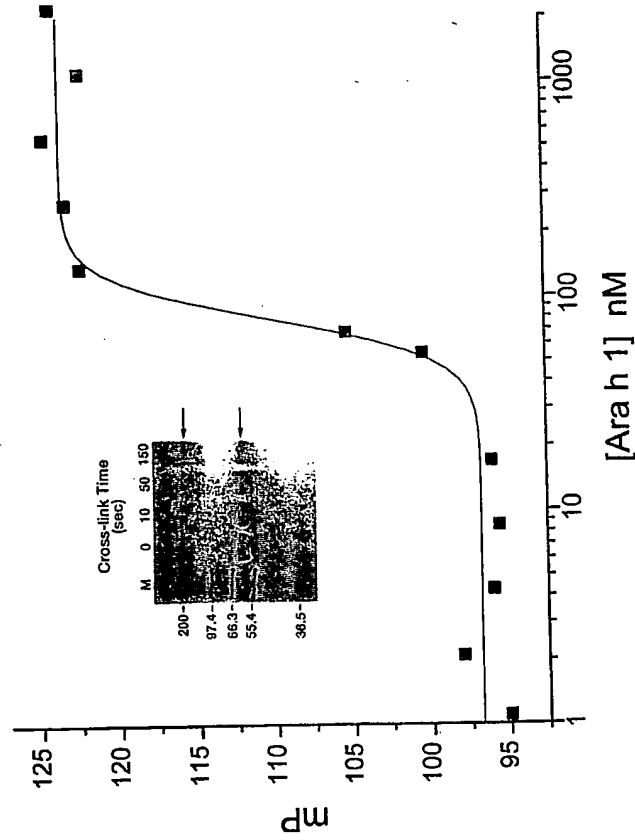


FIG. 6. The Ara h1 allergen forms a stable trimeric structure. Trace fluorescence-labeled Ara h1 was mixed with unlabeled Ara h1, and fluorescence polarization measurements ( $mP$ ) were made at each concentration. Each point represents the average of three different experiments. Samples from the 200 nM concentration were then subjected to cross-linking with constant amounts of DSP for varying lengths of time, and the products were electrophoresed on SDS-polyacrylamide gels. Protein bands were visualized by Coomassie staining. Lower arrow indicates the Ara h1 monomer (~60 kDa), and the upper band represents the Ara h1 trimer (~180 kDa).

557403 64 22 963

# CHYMOTRYPSIN

Panel. A.

Panel. B.

0 —————> 3 hrs 0 —————> 3 hrs

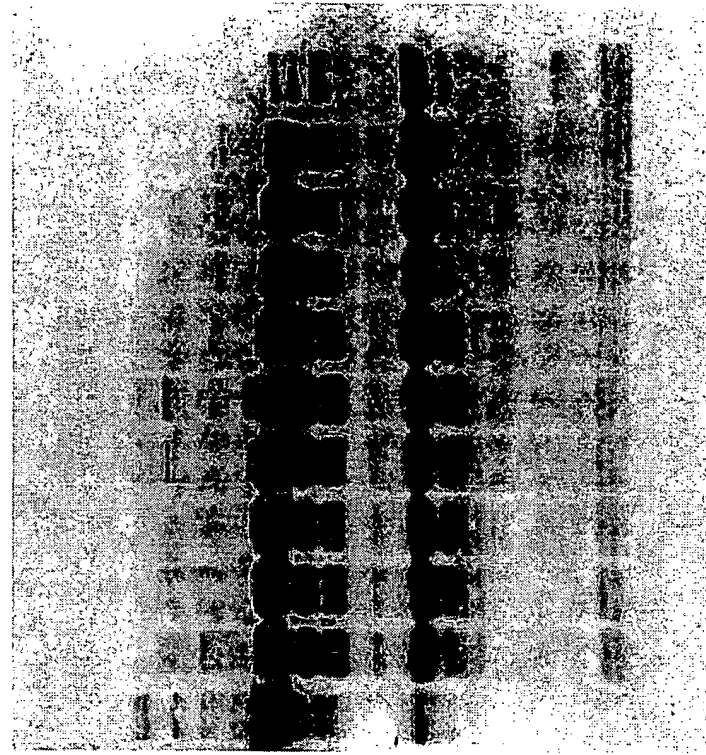
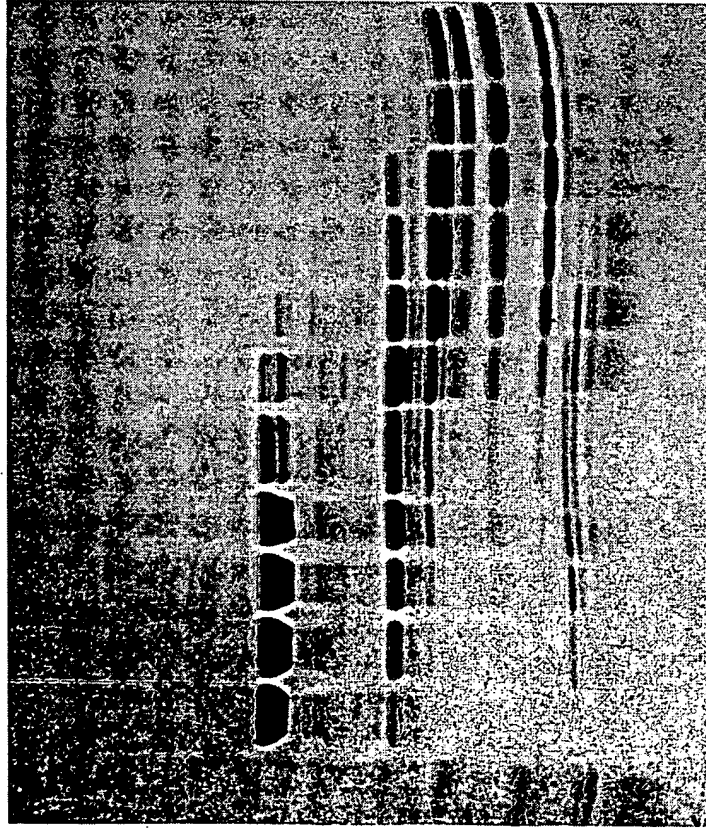


Fig. 7 (A, B)

# CHYMOTRYPSIN

Fig. 7 (c)

Panel C.

0 —————→ 3 hrs

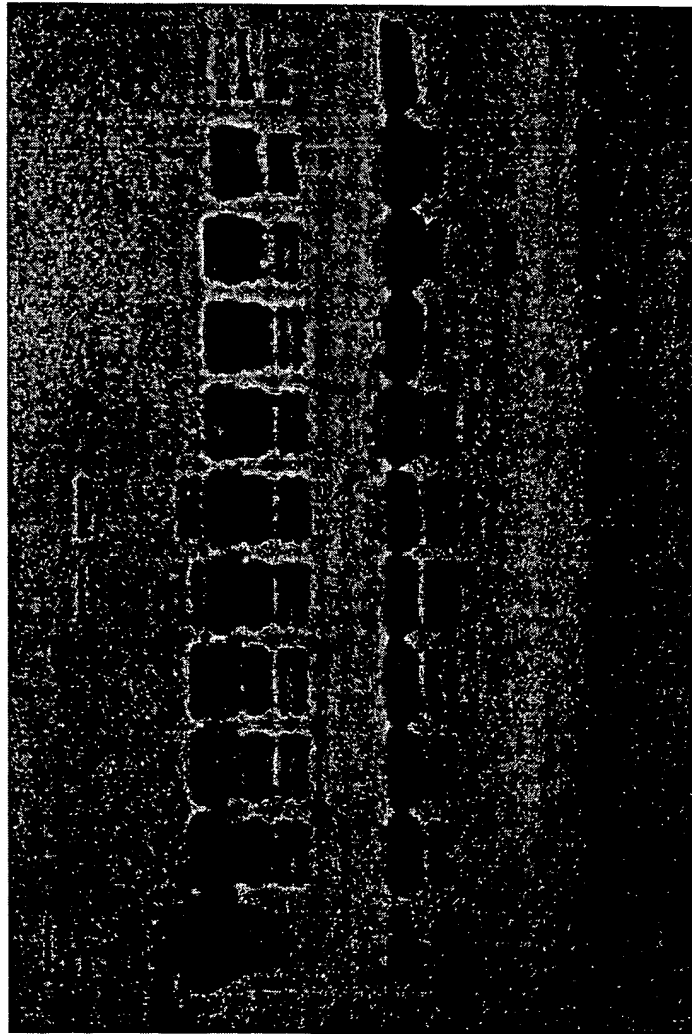
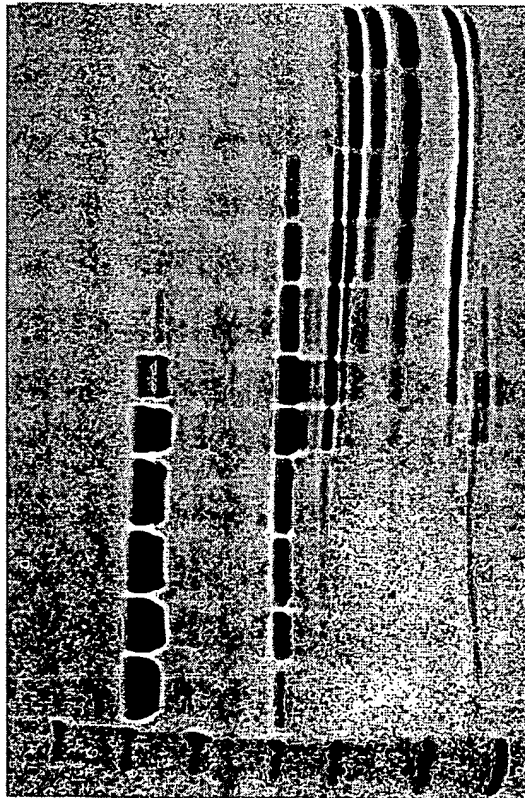


Fig. 7 (c)

TRYPSIN

Panel A.

0 —————→ 3 hrs



Panel B.

0 —————→ 3 hrs

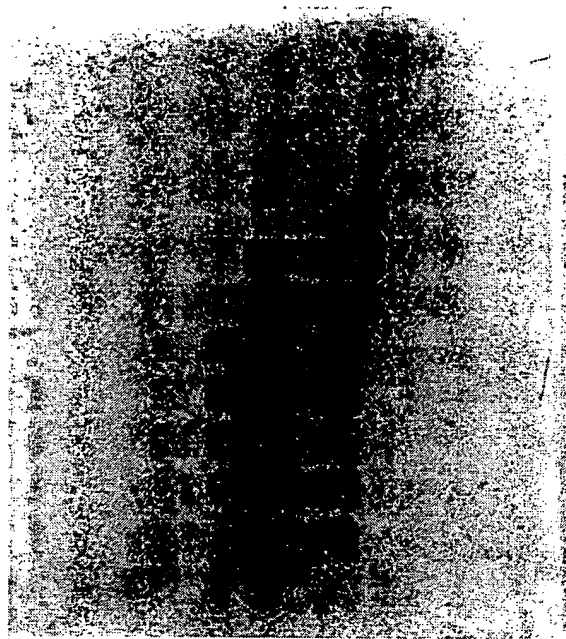
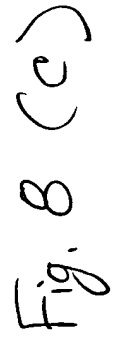


Fig. 8 (A, B)



1992a, 1992b, 1992c, 1992d, 1992e, 1992f, 1992g, 1992h, 1992i, 1992j, 1992k, 1992l, 1992m, 1992n, 1992o, 1992p, 1992q, 1992r, 1992s, 1992t, 1992u, 1992v, 1992w, 1992x, 1992y, 1992z, 1993a, 1993b, 1993c, 1993d, 1993e, 1993f, 1993g, 1993h, 1993i, 1993j, 1993k, 1993l, 1993m, 1993n, 1993o, 1993p, 1993q, 1993r, 1993s, 1993t, 1993u, 1993v, 1993w, 1993x, 1993y, 1993z, 1994a, 1994b, 1994c, 1994d, 1994e, 1994f, 1994g, 1994h, 1994i, 1994j, 1994k, 1994l, 1994m, 1994n, 1994o, 1994p, 1994q, 1994r, 1994s, 1994t, 1994u, 1994v, 1994w, 1994x, 1994y, 1994z, 1995a, 1995b, 1995c, 1995d, 1995e, 1995f, 1995g, 1995h, 1995i, 1995j, 1995k, 1995l, 1995m, 1995n, 1995o, 1995p, 1995q, 1995r, 1995s, 1995t, 1995u, 1995v, 1995w, 1995x, 1995y, 1995z, 1996a, 1996b, 1996c, 1996d, 1996e, 1996f, 1996g, 1996h, 1996i, 1996j, 1996k, 1996l, 1996m, 1996n, 1996o, 1996p, 1996q, 1996r, 1996s, 1996t, 1996u, 1996v, 1996w, 1996x, 1996y, 1996z, 1997a, 1997b, 1997c, 1997d, 1997e, 1997f, 1997g, 1997h, 1997i, 1997j, 1997k, 1997l, 1997m, 1997n, 1997o, 1997p, 1997q, 1997r, 1997s, 1997t, 1997u, 1997v, 1997w, 1997x, 1997y, 1997z, 1998a, 1998b, 1998c, 1998d, 1998e, 1998f, 1998g, 1998h, 1998i, 1998j, 1998k, 1998l, 1998m, 1998n, 1998o, 1998p, 1998q, 1998r, 1998s, 1998t, 1998u, 1998v, 1998w, 1998x, 1998y, 1998z, 1999a, 1999b, 1999c, 1999d, 1999e, 1999f, 1999g, 1999h, 1999i, 1999j, 1999k, 1999l, 1999m, 1999n, 1999o, 1999p, 1999q, 1999r, 1999s, 1999t, 1999u, 1999v, 1999w, 1999x, 1999y, 1999z, 2000a, 2000b, 2000c, 2000d, 2000e, 2000f, 2000g, 2000h, 2000i, 2000j, 2000k, 2000l, 2000m, 2000n, 2000o, 2000p, 2000q, 2000r, 2000s, 2000t, 2000u, 2000v, 2000w, 2000x, 2000y, 2000z, 2001a, 2001b, 2001c, 2001d, 2001e, 2001f, 2001g, 2001h, 2001i, 2001j, 2001k, 2001l, 2001m, 2001n, 2001o, 2001p, 2001q, 2001r, 2001s, 2001t, 2001u, 2001v, 2001w, 2001x, 2001y, 2001z, 2002a, 2002b, 2002c, 2002d, 2002e, 2002f, 2002g, 2002h, 2002i, 2002j, 2002k, 2002l, 2002m, 2002n, 2002o, 2002p, 2002q, 2002r, 2002s, 2002t, 2002u, 2002v, 2002w, 2002x, 2002y, 2002z, 2003a, 2003b, 2003c, 2003d, 2003e, 2003f, 2003g, 2003h, 2003i, 2003j, 2003k, 2003l, 2003m, 2003n, 2003o, 2003p, 2003q, 2003r, 2003s, 2003t, 2003u, 2003v, 2003w, 2003x, 2003y, 2003z, 2004a, 2004b, 2004c, 2004d, 2004e, 2004f, 2004g, 2004h, 2004i, 2004j, 2004k, 2004l, 2004m, 2004n, 2004o, 2004p, 2004q, 2004r, 2004s, 2004t, 2004u, 2004v, 2004w, 2004x, 2004y, 2004z, 2005a, 2005b, 2005c, 2005d, 2005e, 2005f, 2005g, 2005h, 2005i, 2005j, 2005k, 2005l, 2005m, 2005n, 2005o, 2005p, 2005q, 2005r, 2005s, 2005t, 2005u, 2005v, 2005w, 2005x, 2005y, 2005z, 2006a, 2006b, 2006c, 2006d, 2006e, 2006f, 2006g, 2006h, 2006i, 2006j, 2006k, 2006l, 2006m, 2006n, 2006o, 2006p, 2006q, 2006r, 2006s, 2006t, 2006u, 2006v, 2006w, 2006x, 2006y, 2006z, 2007a, 2007b, 2007c, 2007d, 2007e, 2007f, 2007g, 2007h, 2007i, 2007j, 2007k, 2007l, 2007m, 2007n, 2007o, 2007p, 2007q, 2007r, 2007s, 2007t, 2007u, 2007v, 2007w, 2007x, 2007y, 2007z, 2008a, 2008b, 2008c, 2008d, 2008e, 2008f, 2008g, 2008h, 2008i, 2008j, 2008k, 2008l, 2008m, 2008n, 2008o, 2008p, 2008q, 2008r, 2008s, 2008t, 2008u, 2008v, 2008w, 2008x, 2008y, 2008z, 2009a, 2009b, 2009c, 2009d, 2009e, 2009f, 2009g, 2009h, 2009i, 2009j, 2009k, 2009l, 2009m, 2009n, 2009o, 2009p, 2009q, 2009r, 2009s, 2009t, 2009u, 2009v, 2009w, 2009x, 2009y, 2009z, 2010a, 2010b, 2010c, 2010d, 2010e, 2010f, 2010g, 2010h, 2010i, 2010j, 2010k, 2010l, 2010m, 2010n, 2010o, 2010p, 2010q, 2010r, 2010s, 2010t, 2010u, 2010v, 2010w, 2010x, 2010y, 2010z, 2011a, 2011b, 2011c, 2011d, 2011e, 2011f, 2011g, 2011h, 2011i, 2011j, 2011k, 2011l, 2011m, 2011n, 2011o, 2011p, 2011q, 2011r, 2011s, 2011t, 2011u, 2011v, 2011w, 2011x, 2011y, 2011z, 2012a, 2012b, 2012c, 2012d, 2012e, 2012f, 2012g, 2012h, 2012i, 2012j, 2012k, 2012l, 2012m, 2012n, 2012o, 2012p, 2012q, 2012r, 2012s, 2012t, 2012u, 2012v, 2012w, 2012x, 2012y, 2012z, 2013a, 2013b, 2013c, 2013d, 2013e, 2013f, 2013g, 2013h, 2013i, 2013j, 2013k, 2013l, 2013m, 2013n, 2013o, 2013p, 2013q, 2013r, 2013s, 2013t, 2013u, 2013v, 2013w, 2013x, 2013y, 2013z, 2014a, 2014b, 2014c, 2014d, 2014e, 2014f, 2014g, 2014h, 2014i, 2014j, 2014k, 2014l, 201



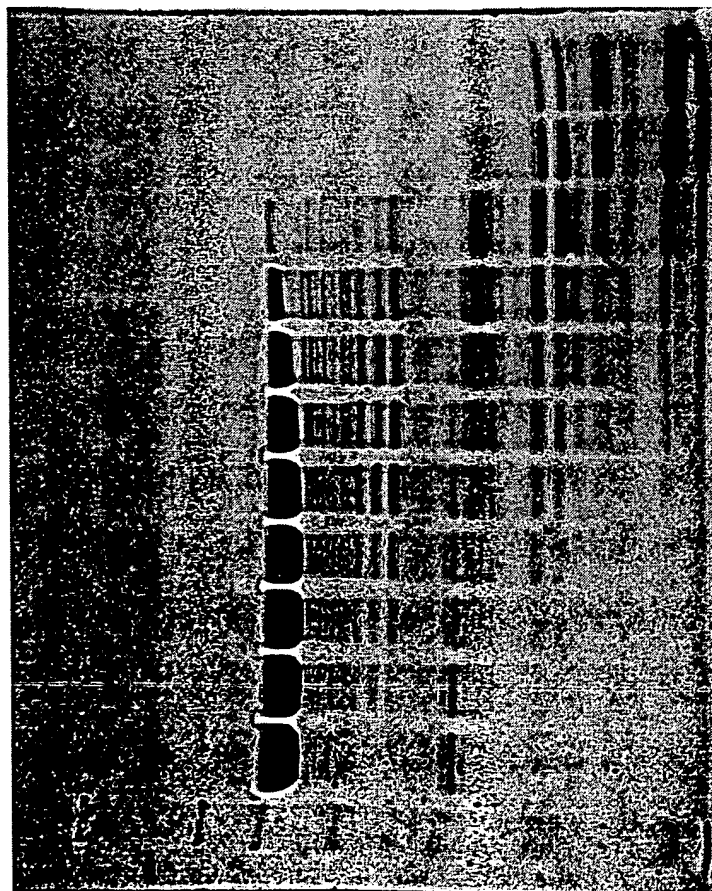
Li<sub>2</sub>O

66769-672969

# Pepsin

Panel A

0 → 3 hrs



Panel B

0 → 3 hrs

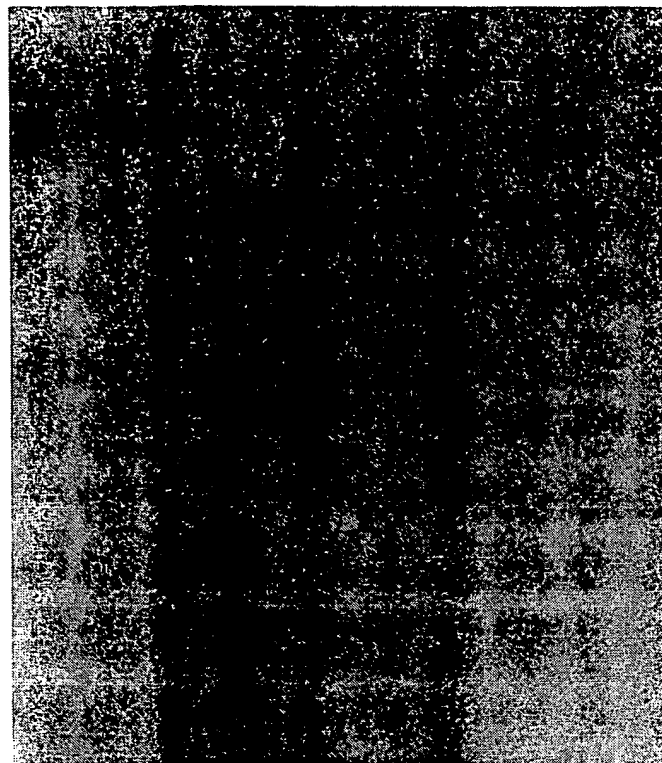


Fig. 9

# MyoD Digestion

100 100 100 100 100 100 100 100 100 100

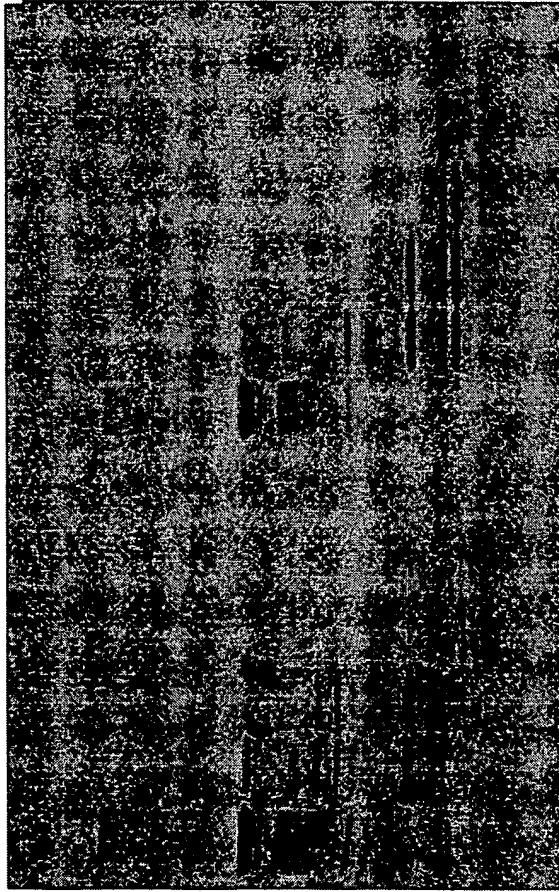


Fig. 10

### Ara h 3 Amino Acid Sequence

1	I	S	F	R	Q	Q	P	E	E	N	A	C	Q	F	Q	R	L	N	A	Q	R	P	D	N	R	I	E
28	S	E	G	G	Y	I	E	T	W	N	P	N	N	Q	E	F	E	C	A	G	V	A	L	S	R	L	V
54	L	R	R	N	A	L	R	R	P	F	Y	S	N	A	P	Q	E	I	F	I	Q	Q	G	R	G	Y	F
82	G	L	I	F	P	G	C	P	R	H	Y	E	E	P	H	T	Q	G	R	R	S	Q	S	Q	R	P	P
109	R	R	L	Q	G	E	D	Q	S	Q	Q	Q	R	D	S	H	Q	K	V	H	R	F	D	E	G	D	L
136	I	A	V	P	T	G	V	A	F	W	L	Y	N	D	H	D	T	D	V	V	A	V	S	L	T	D	T
163	N	N	N	D	N	Q	L	D	Q	F	P	R	R	F	N	L	A	G	N	T	E	Q	E	F	E	F	L
190	R	Y	Q	Q	Q	S	R	Q	S	R	R	R	S	L	P	Y	S	P	Y	S	P	Q	S	Q	P	R	Q
207	E	E	R	E	F	S	P	R	G	Q	H	S	R	R	E	R	A	G	Q	E	E	E	N	E	G	G	N
234	I	F	S	G	F	T	P	E	F	L	E	Q	A	F	Q	V	D	D	R	Q	I	V	Q	N	L	R	G
261	E	T	E	S	E	E	E	G	A	I	V	T	V	R	G	G	L	R	I	L	S	P	D	R	K	R	R
288	A	D	E	E	E	E	Y	D	E	D	E	Y	E	Y	D	E	E	D	R	R	R	G	R	G	S	R	G
315	R	G	N	G	I	E	E	T	I	C	T	A	S	A	K	K	N	I	G	R	N	R	S	P	D	I	Y
342	N	P	Q	A	G	S	L	K	T	A	N	D	L	N	L	L	I	L	R	W	L	G	L	S	A	E	Y
369	G	N	L	Y	R	N	A	L	F	V	A	H	Y	N	T	N	A	H	S	I	I	Y	R	L	R	G	R
396	A	H	V	Q	V	V	D	S	N	G	N	R	V	Y	D	E	E	L	Q	E	G	H	V	L	V	V	P
423	Q	N	F	A	V	A	G	K	S	Q	S	E	N	F	E	Y	V	A	F	K	T	D	S	R	P	S	I
450	A	N	L	A	G	E	N	S	V	I	D	N	L	P	E	E	V	V	A	N	S	Y	G	L	Q	R	E
477	Q	A	R	Q	L	K	N	N	N	P	F	K	F	F	V	P	P	S	Q	Q	S	P	R	A	V	A	

Fig. 11

# A.

	51					100
Ara h 3	SEGGYIETWN	PNNQEFECAG	VALSRLVLRR	NALRRFYSN	APQEIFIQQG	
G1 Soy	SEGGLIETWN	PNNKPFQAG	VALSRCTLNR	NALRRSYTN	GPQEIIYQQG	
G2 Soy	SEGFIETWN	PNNKPFQAG	VALSRCTLNR	NALRRSYTN	GPQEIIYQQG	
A2 Pea	SEGGLIETWN	PNNKQFRAG	VALSRATLQH	NALRRPYYSN	APQEIFIQQG	

	101					150
Ara h 3	RGYFGLIFPG	QPRHYEEPHT	QGRRSQSQR	PRRLQGEDQS	QQQRDSHQKV	
G1 Soy	KGIFGMIYPG	CPSTFEFPQQ	PQQRGQSSRP	.....	...QDRHQKI	
G2 Soy	NGIFGMIFPG	CPSTYQEPQE	SQQRGRSQR	.....	...QDRHQKV	
A2 Pea	NGYFGMVFP	CPETFEFPQE	SEQ.GEGRRY	.....	...RDRHQKV	

# B.

	351					400
Ara h 3	EYDEDEY...	.....EYDEE	DRRRGRGSRG	R.....GNG	IEETICTASA	
G1 Soy	EEEEDEKP..	...QCKGKDK	HCQRPRGSQS	KSRR...NG	IDETICTMRL	
G2 Soy	DDDEEEQP..	...QCVETDK	GCQR....QS	KRSR...NG	IDETICTMRL	
A2 Pea	DEDEERQPRH	QRRRGEEEE	DKKERRGSQK	GKSRRQGDNG	LEETVCTAKL	

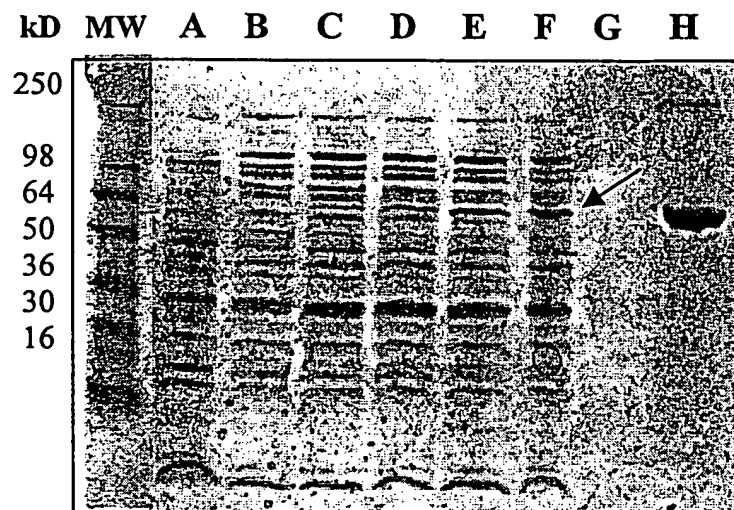
	401					450
Ara h 3	KKNIGRNRSP	DIYNPQAGSL	KTANDLNLLI	ERWLGLSAEY	GNLYRNALFV	
G1 Soy	RHNIGQTSSP	DIYNPQAGSV	TTATSLDFPA	ESWLRLSAEF	GSLRKNAMEFV	
G2 Soy	RQNIGQNSSP	DIYNPQAGSI	TTATSLDFPA	EWLLKLSAQY	GSLRKNAMEFV	
A2 Pea	RLNIGPSSSP	DIYNPEAGRI	KTVTSLDLFV	ERWLKLSAEH	GSLHKNAMEFV	

	451					500
Ara h 3	AHYNTVAHSI	IYRLGRAHV	QVYDSNGNRV	YDEELQECHV	LVPONFAVA	
G1 Soy	PHYNLVANSI	IYALNGRALI	QVYNCNGERV	FDGELQEGRV	LIVPONFVVA	
G2 Soy	PHYTLVANSI	IYALNGRALV	QVYNCNGERV	FDGELQEGV	LIVPONFAVA	
A2 Pea	PHYNLVANSI	IYALKGRARL	QVYNCNGNTV	FDGELEAGRA	LTVPNYAVA	

Fig. 12

**A. Bacterial Expression of Recombinant Ara h 3**



**B. Immunoblot Analysis of Total Bacterial Extract**

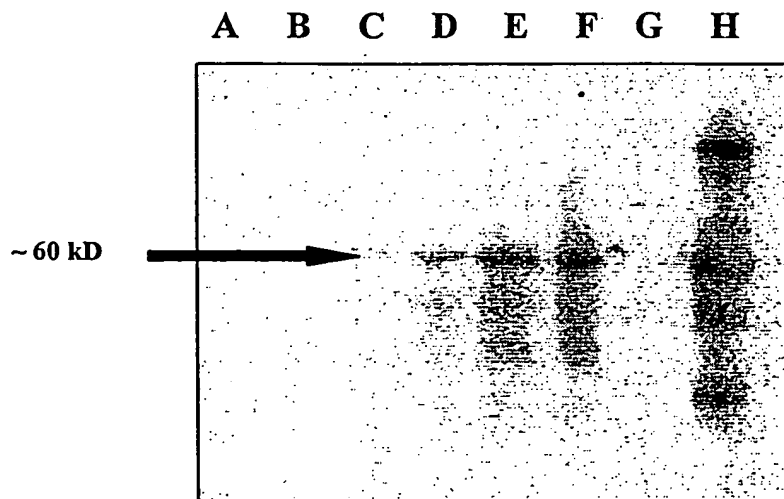


Fig. 13

A.

1 2 3 4 5 6 7 8

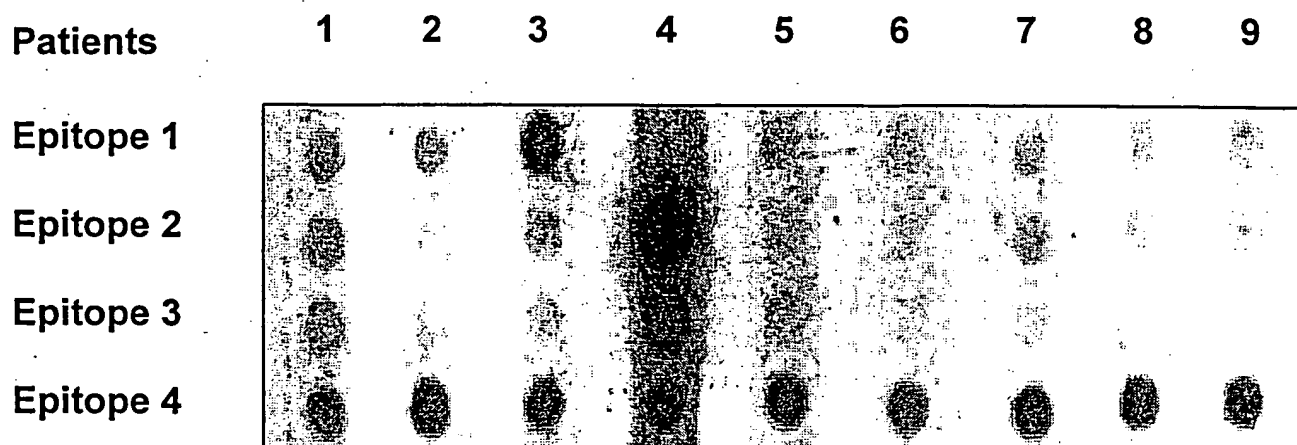


B.

	LSAEYGNLYRNA	LFVAHYNTNAHS
1.	LSAEYGNLYR	
2.	AEYGNLYRNA	
3.	YGNLYRNA	LF
4.	NLYRNA	LFVAHY
5.	YRNA	LFVAHY
6.		NAFVAHYNT
7.		LFVAHYNTNA
8.		VAHYNTNAHS

Fig. 14

### A. Epitope 4 is an Immunodominant Epitope



4

### B. Percentage of Recognition for Each Epitope

<u>Epitope</u>	<u>Sequence</u>	<u>Position</u>	<u>Percentage</u>
1	EQEFLRYQQQ	183-192	5% (1/20)
2	FTPEFLEQAF	246-255	25% (5/20)
3	EYDYDEEDRR	300-309	35% (7/20)
4	LYRNALFVAH	379-388	100% (20/20)

Fig. 15



Peptide 2

F246A

T247A

P248A

E249A

F250A

L251A

E252A

Q253A

A254L

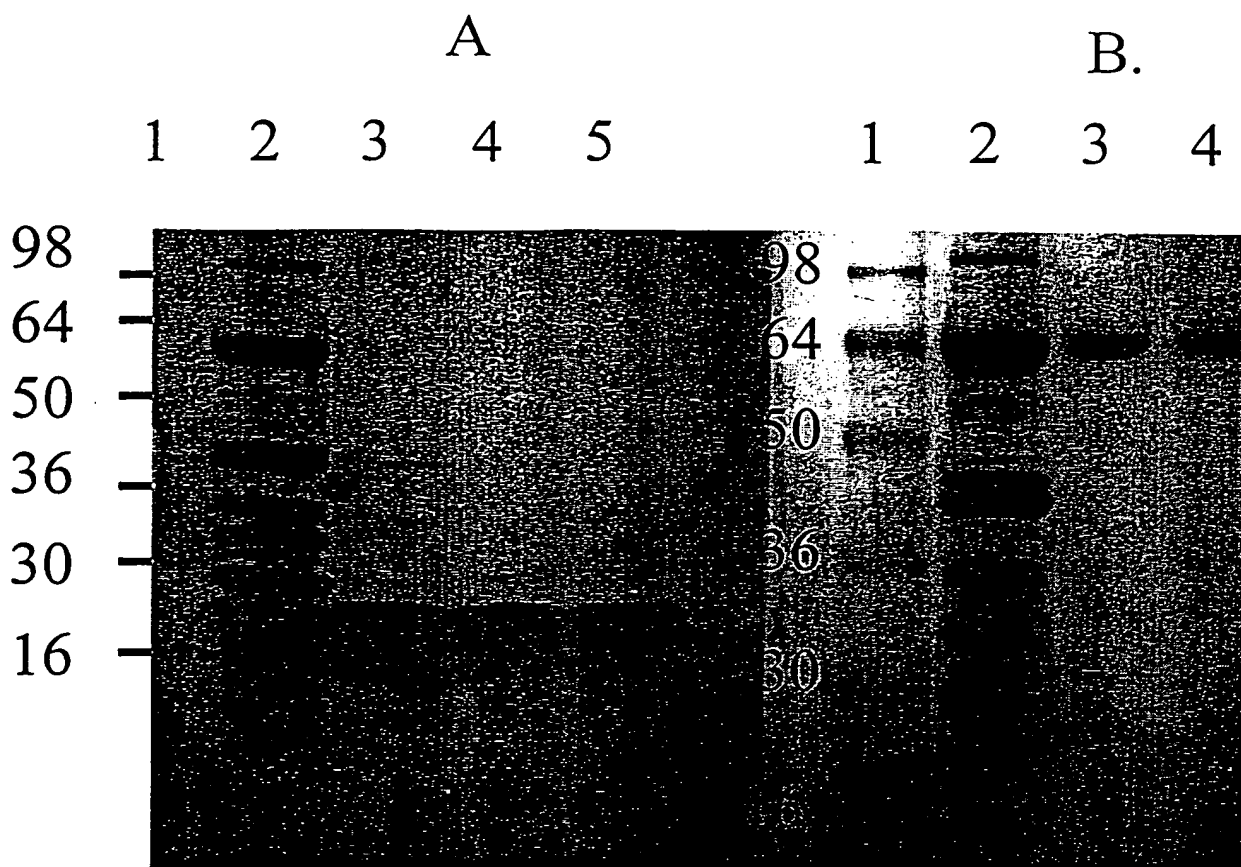
F255A

WT



Fig. 16

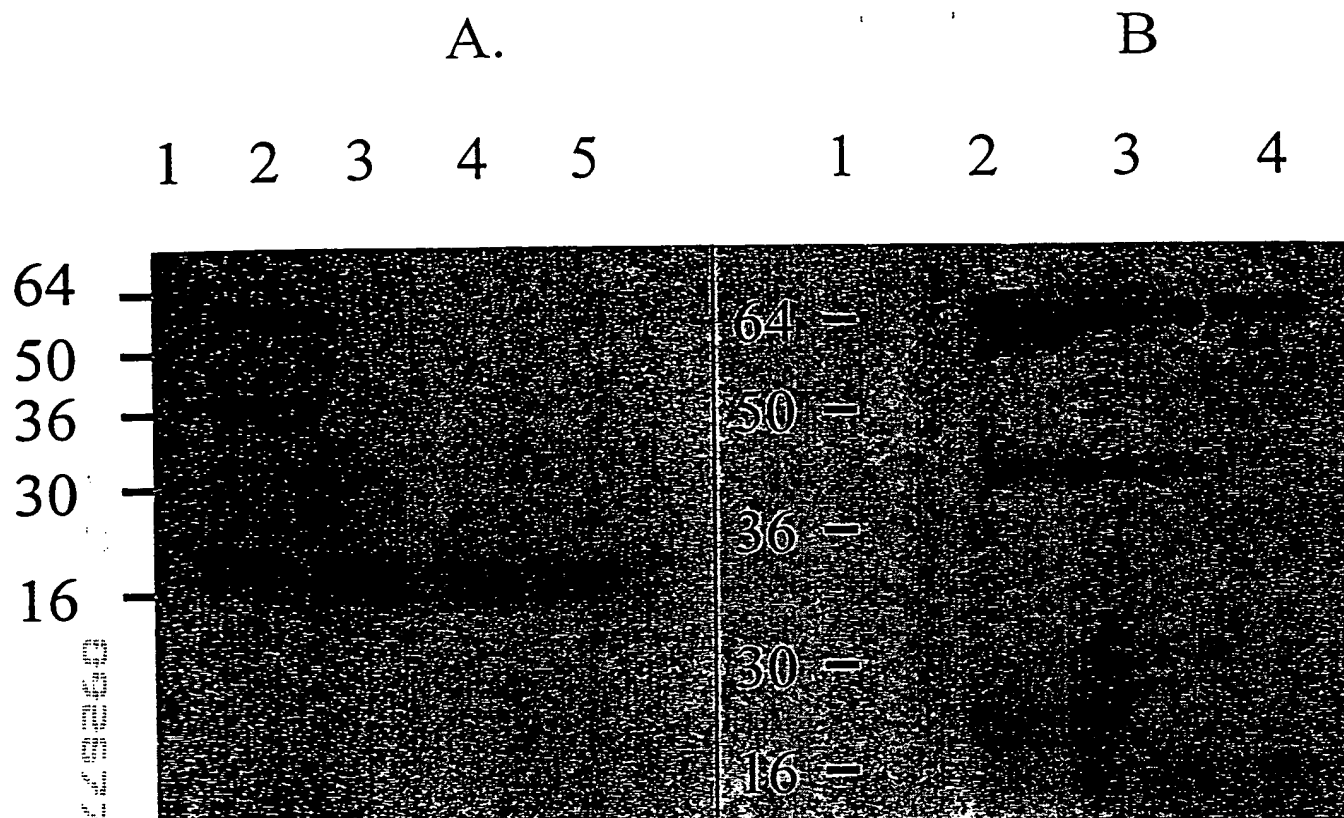
65 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100



**SDS-PAGE analysis of the protein profiles at various stages of allergen purification.**

Panels A and B are the protein profiles resulting from the purification of Ara h 1 and Ara h 2, respectively. Lanes are as follows: *lanes A1 and B1*, protein standards; *lanes A2 and B2*, crude peanut extract; *lane A3*, 25% ammonium sulfate pellet; *A4*, Ara h 2 fraction following anion exchange chromatography; *lane A5*, Ara h 2 fraction following hydrophobic chromatography *lane B3*, 100% ammonium sulfate pellet; *lane B4*, Ara h 1 fraction following cation exchange chromatography.

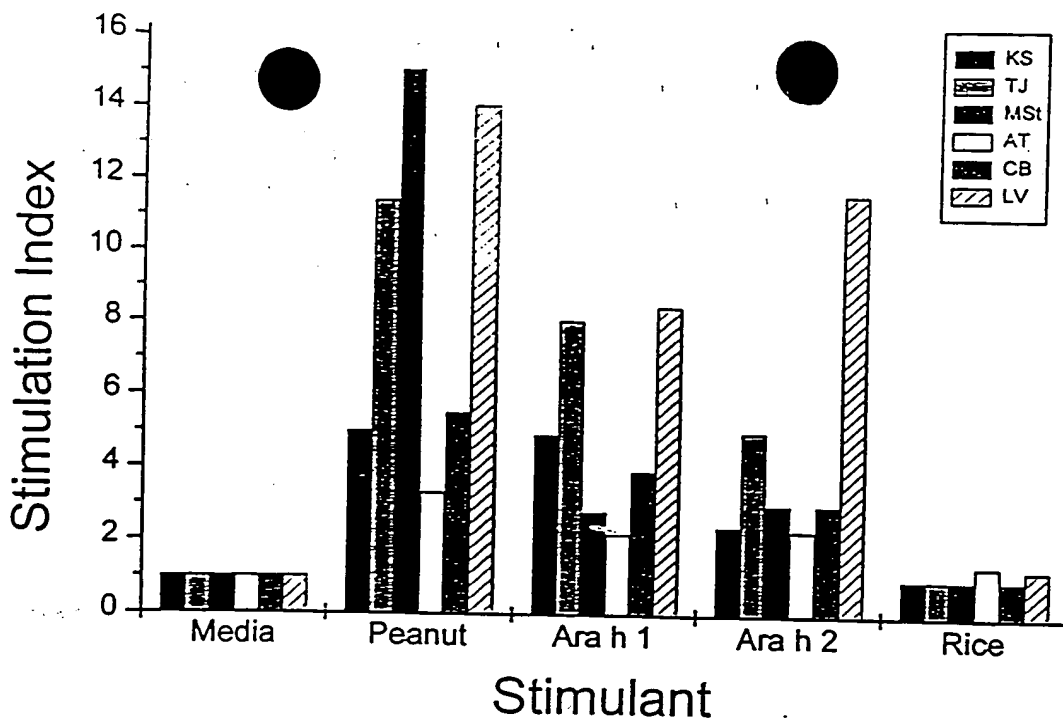
Fig. 17



**Immunoblot of the purified proteins using serum IgE from allergic individuals.**

Panels A and B are the protein profiles resulting from the purification of Ara h 1 and Ara h 2 that were blotted to nitrocellulose and detected by western blot analysis using serum IgE from allergic individuals as the primary antibody. Lanes are as follows: lanes A1 and B1, protein standards; lanes A2 and B2, crude peanut extract; lane A3, 25% ammonium sulfate pellet; A4, Ara h 2 fraction following anion exchange chromatography; lane A5, Ara h 2 fraction following hydrophobic chromatography lane B3, 100% ammonium sulfate pellet; lane B4, Ara h 1 fraction following cation exchange chromatography.

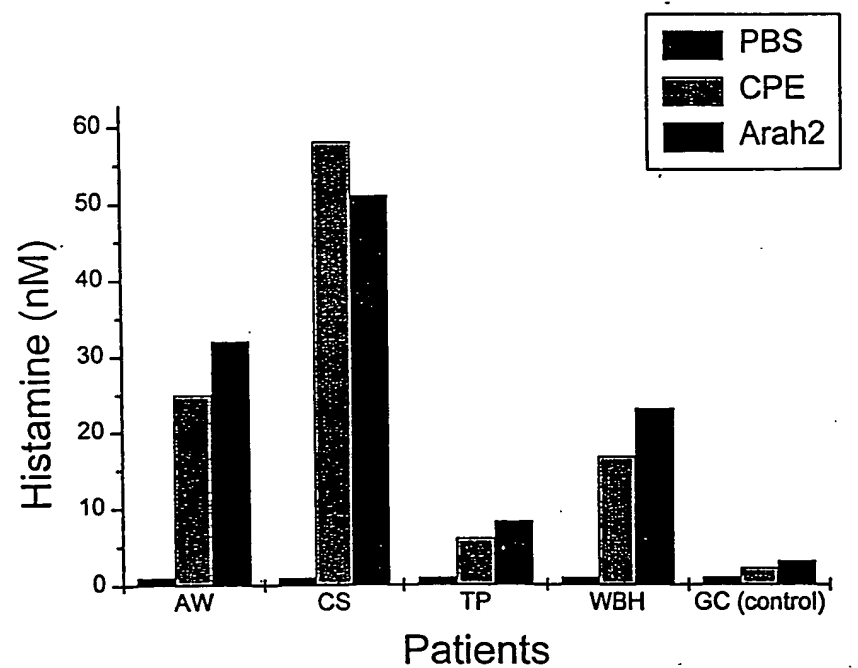
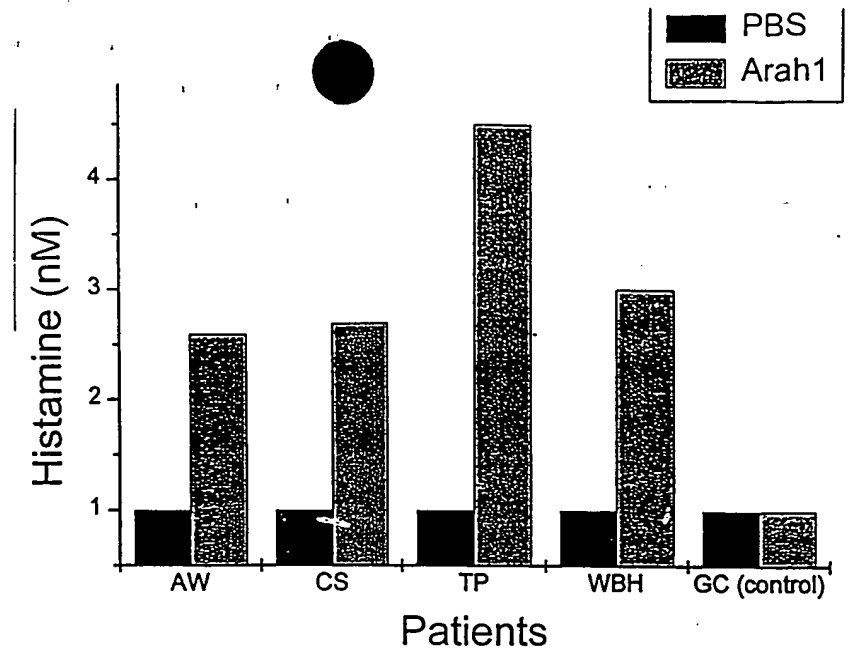
Fig. 18



Purified Ara h 1 and Ara h 2 can stimulate T cell proliferation.

T cells were isolated from peanut allergic individuals and placed into 96 well plates at  $4 \times 10^4$  cells/well and treated in triplicates with media, crude peanut extracts (positive control), Ara h 1, Ara h 2 or rice extracts (negative control). The cells were allowed to proliferate for 6 days and then incubated with  $^3\text{H}$ -thymidine ( $1\mu\text{Ci}/\text{well}$ ) at  $37^\circ\text{C}$  for 6-8 hrs and then harvested onto glass fiber filters. T-cell proliferation was estimated by quantitating the amount of  $^3\text{H}$ -thymidine incorporation into the DNA of proliferating cells.  $^3\text{H}$ -thymidine incorporation is reported as stimulation (SI) above media treated control cells.

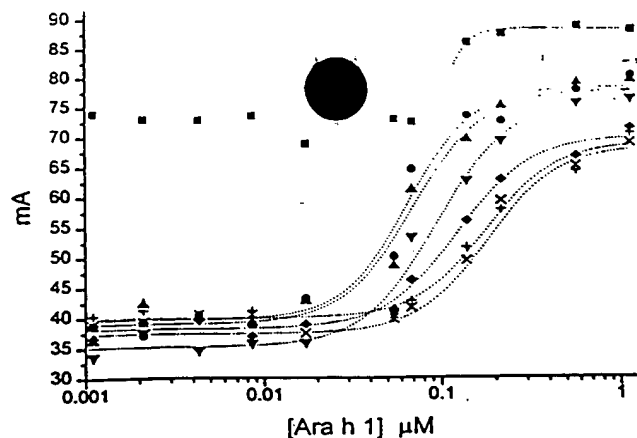
Fig. 19



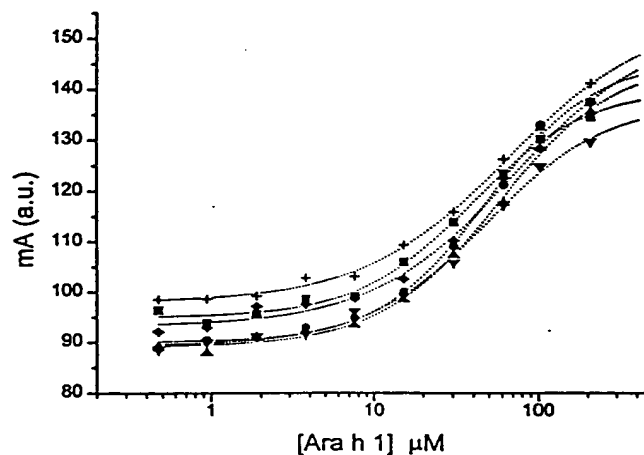
**Purified Ara h 1 and Ara h 2 can stimulate histamine release from mast cells of peanut allergic individuals.**

Mast cells from whole blood of allergic individuals (on the x-axis) from left to right were treated with PBS (negative control), crude peanut extracts (positive control), Ara h 1 (panel A) or Ara h 2 (panel B). The release of histamine is reported on the y-axis in nM. The histamine release assay was that developed by Immunologics (City, State) and it was performed exactly as described by the manufacturer.

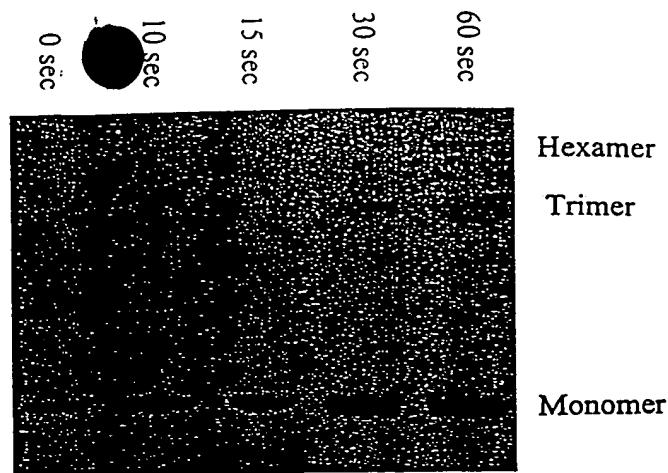
Fig. 20



**Panel A:** The formation of trimers at low concentrations of Ara h 1. The samples were in binding buffer plus various concentrations of NaCl as indicated: ○ 0 mM NaCl, □ 100 mM NaCl, ● 300 mM NaCl, ■ 500 mM NaCl, ▲ 900 mM NaCl, △ 1400 mM NaCl, × 1800 mM NaCl.



**Panel B:** The formation of hexamers at high concentrations of Ara h 1. The samples were in binding buffer plus various concentrations of NaCl as indicated: ○ 100 mM NaCl, ● 400 mM NaCl, ■ 600 mM NaCl, ▲ 800 mM NaCl, △ 1100 mM NaCl, × 1300 mM NaCl, \* 1800 mM NaCl.

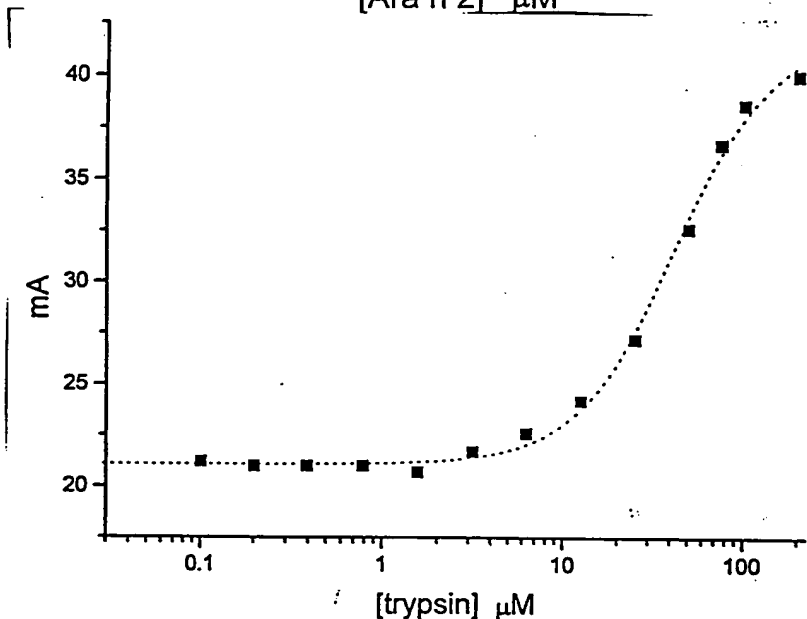
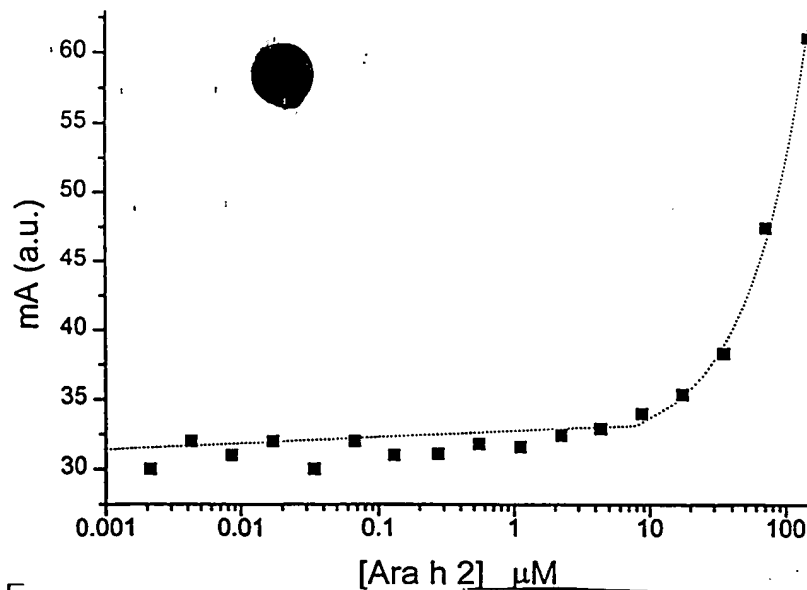


**Panel C:** Samples from the 80 μM concentration from Panel A were subjected to limited cross-linking at various time points and the products electrophoresed on SDS polyacrylamide gels. Protein bands were visualized by Coomassie staining. Lower arrow indicates the Ara h 1 monomer (~60 kDa), the next highest band represents the Ara h 1 trimer (~180 kDa), and the highest molecular weight band represents Ara h 1 hexamer (~360 kDa).

The purified Ara h 1 protein retains its native structure as indicated by its ability to form homotrimers and hexamers.

Fluorescence anisotropy was used to follow the formation of Ara h 1 higher order structure. All fluorescence measurements were made using a Beacon fluorescence polarization spectrometer (Pan Vera, Madison, WI) with fixed excitation (490 nm) and emission (530 nm) wavelengths which are specified for fluorescein use. Fluorescence measurements were done at room temperature (24 °C) in binding buffer (50 mM Tris, 1 mM EDTA, 100 mM NaCl, 2 mM DTT, 5% glycerol, pH 7.5) in a final volume of 1.1 ml. A constant amount of fluorescein labeled protein (10 nM of Ara h 1) was diluted with binding buffer and mixed with various concentrations of unlabeled Ara h 1 to analyze homo-oligomer formation. Serial dilutions of the desalted proteins (by 0.5 or 0.8 increments) were made in binding buffer and the appropriate amounts were added to constant amounts of fluorescein-labeled protein. Each data point is an average of three independent measurements. The intensity of fluorescence remained constant throughout the anisotropy measurements.

Fig. 21



Ara h 2 does not form homo-oligomers but does bind to trypsin.

The fluorescence milli-anisotropy (mA) of fluorescent-labeled Ara h 2 titrated with unlabeled Ara h 2 or trypsin at different concentrations is measured and plotted. All fluorescent measurements were performed exactly as described in Fig. 5.

**Panel A:** The fluorescence milli-anisotropy (mA) of fluorescent-labeled Ara h 2 titrated with unlabeled Ara h 2 at different concentrations is measured and plotted versus the concentration of unlabeled Ara h 2. The samples were in binding buffer plus ( ) 100 mM NaCl

**Panel B:** The fluorescence milli-anisotropy (mA) of fluorescent-labeled Ara h 2 titrated with unlabeled trypsin at different concentrations is measured and plotted versus the concentration of unlabeled trypsin. The samples were in binding buffer plus ( ) 100 mM NaCl.

Fig. 22

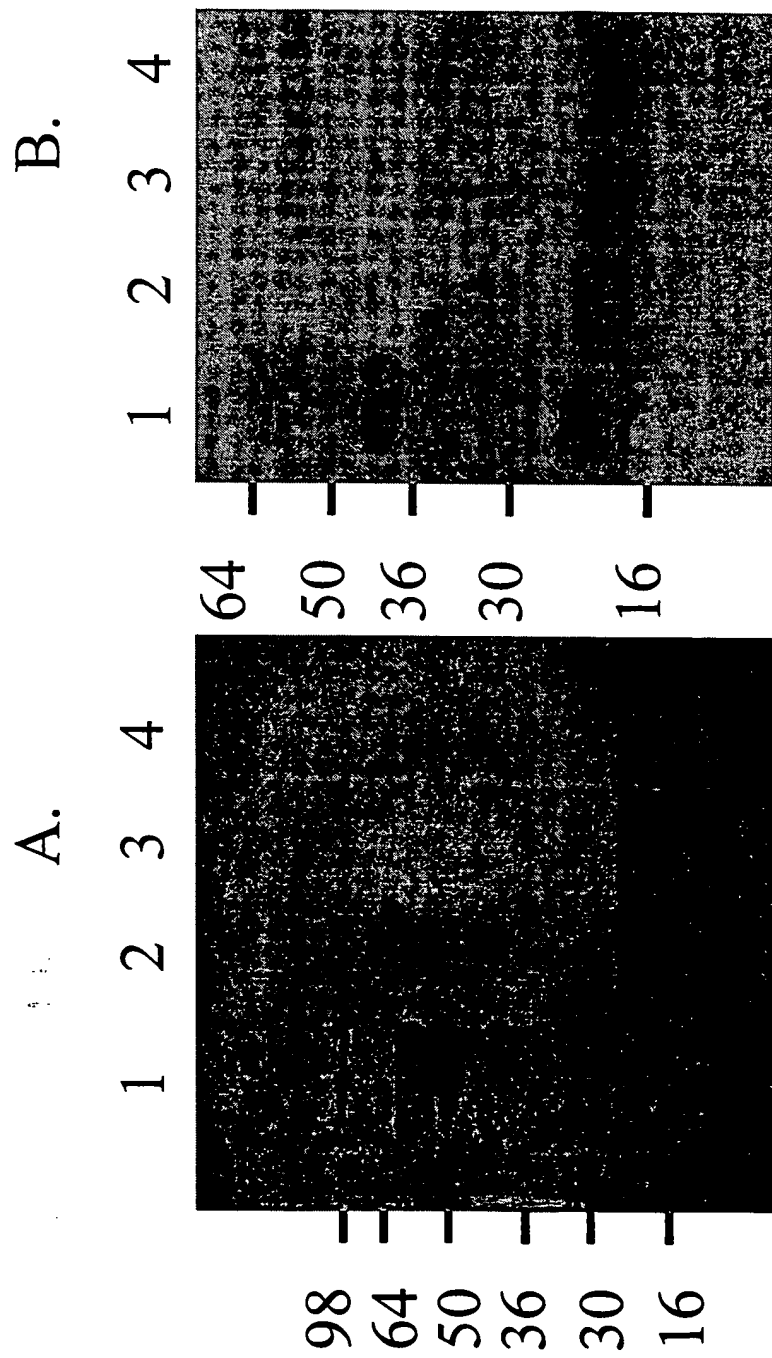
[illegible]

Fig. 23



Anti-Zap-70 Ip, Anti-TCR western

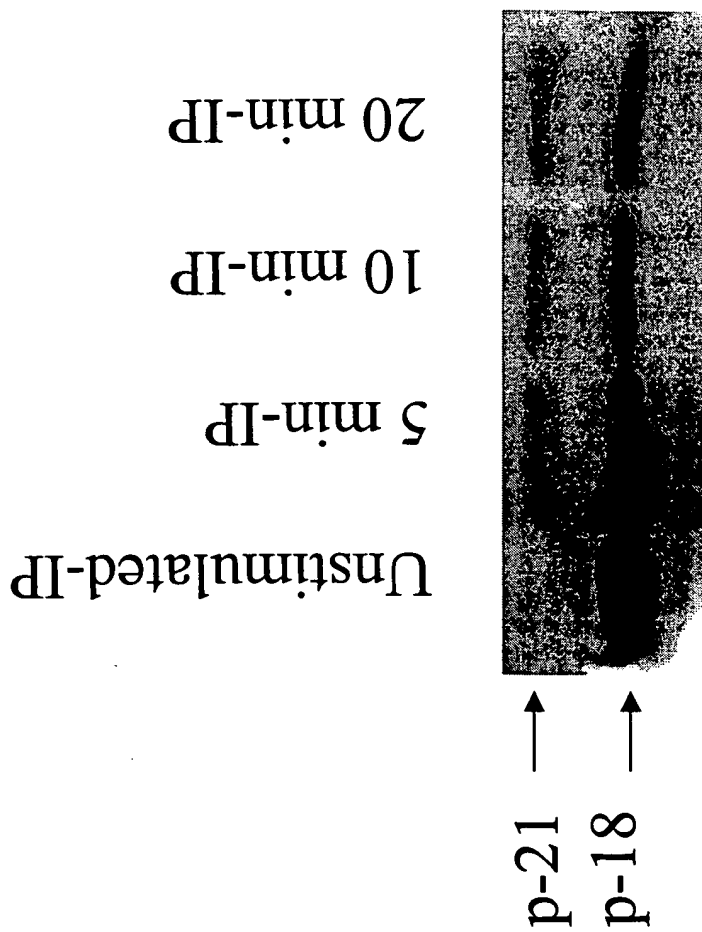


Fig. 24A

Anti-Zap-70-IP, Anti-pTyr western.

Unstimulated-IP  
5 min-IP  
10 min-IP  
20 min-IP

p-21  
p-18

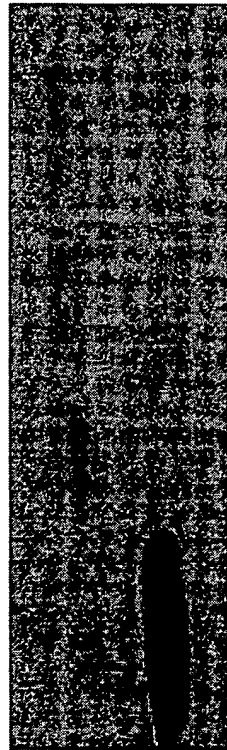


Fig. 24B

65 4 4 0 3 4 2 2 6 0

Anti-TCR-IP, Anti-ERK-1 western

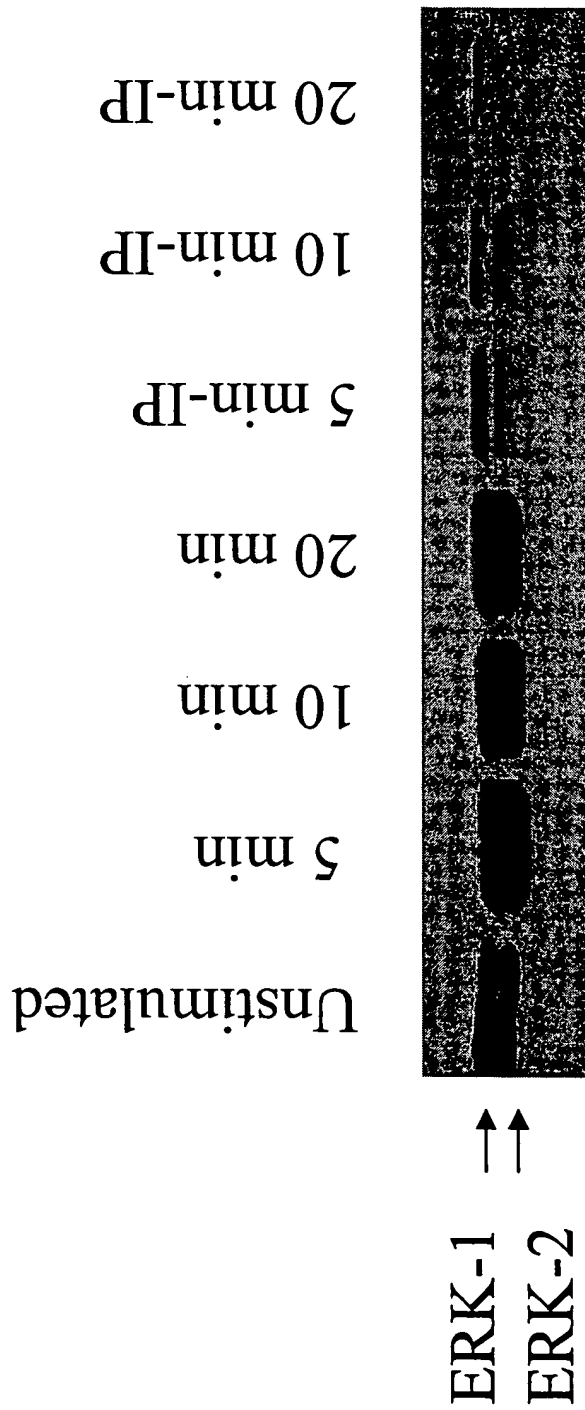
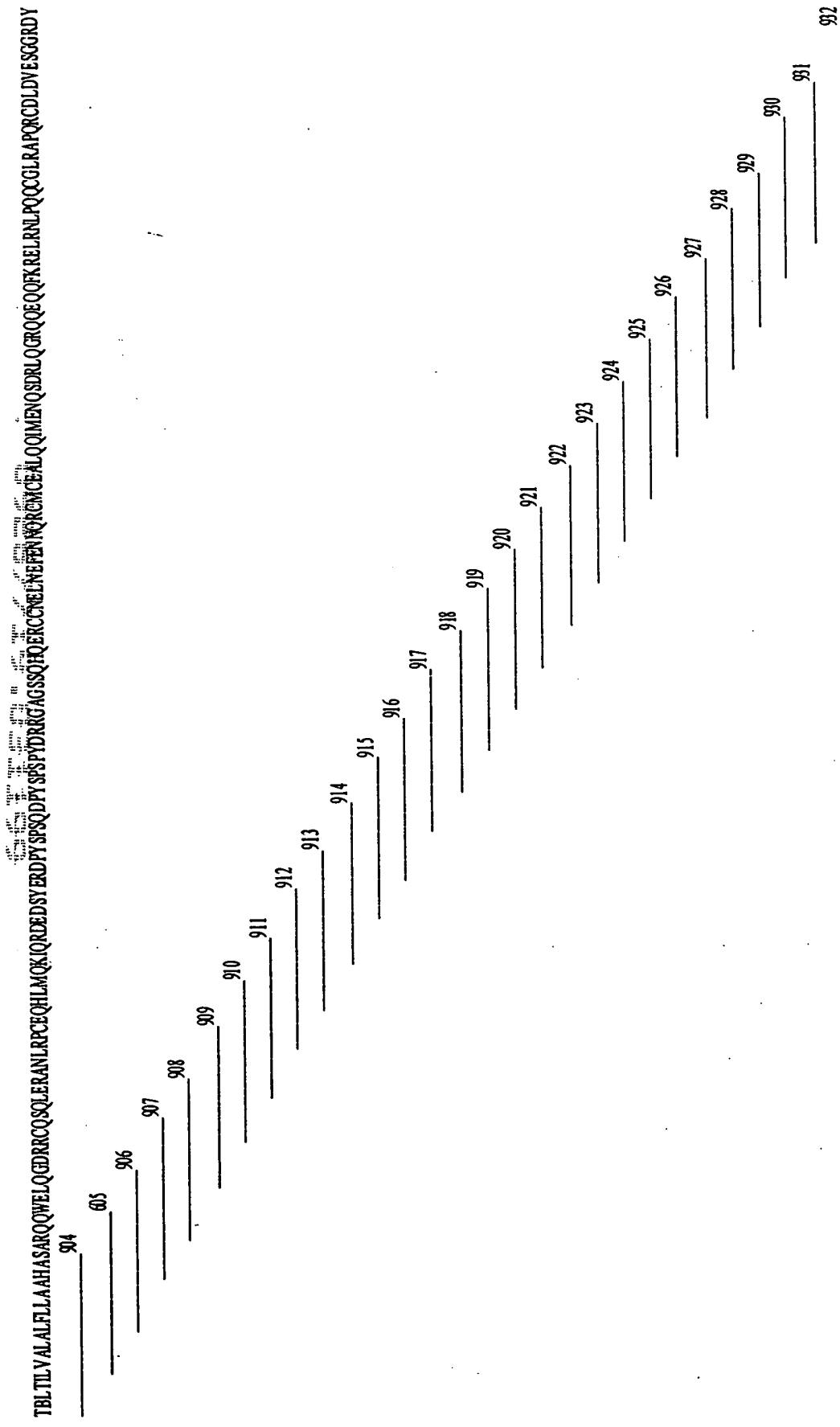


Fig. 24C

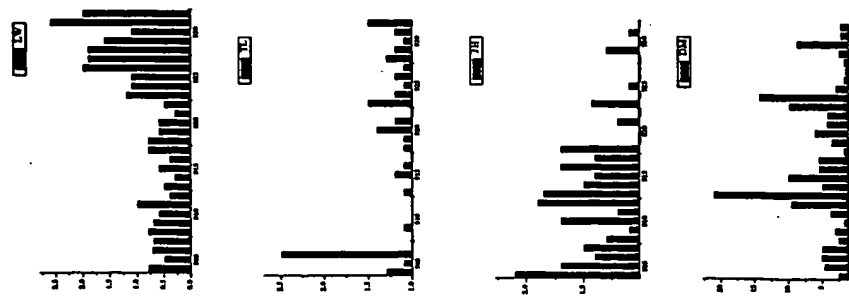
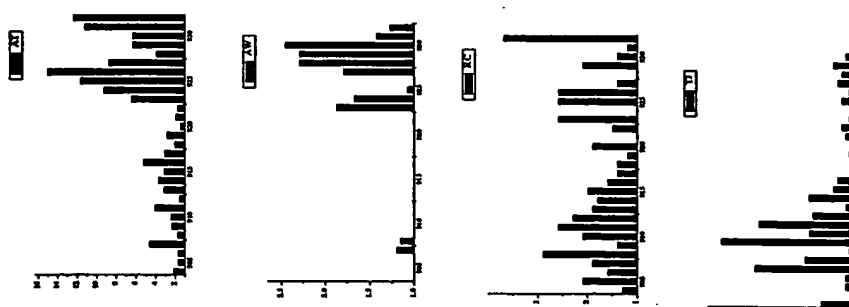
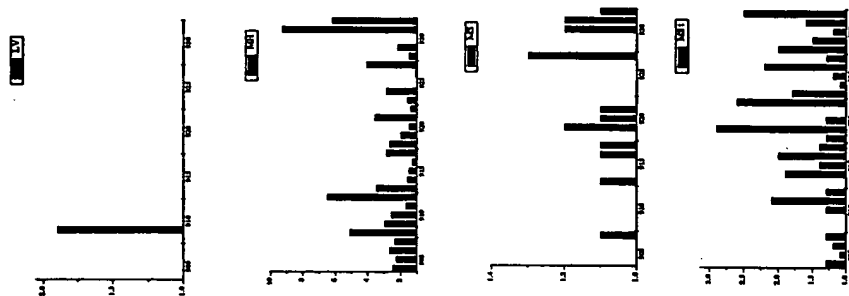
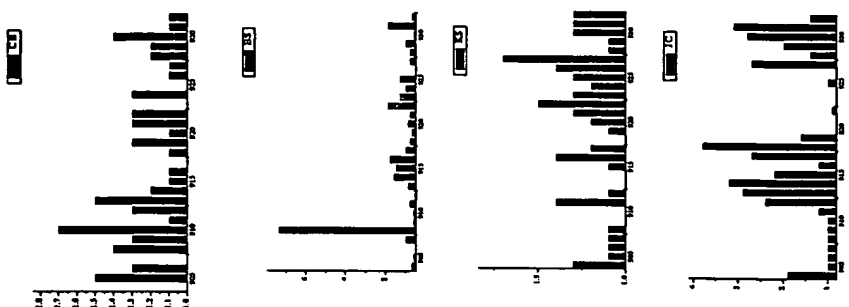
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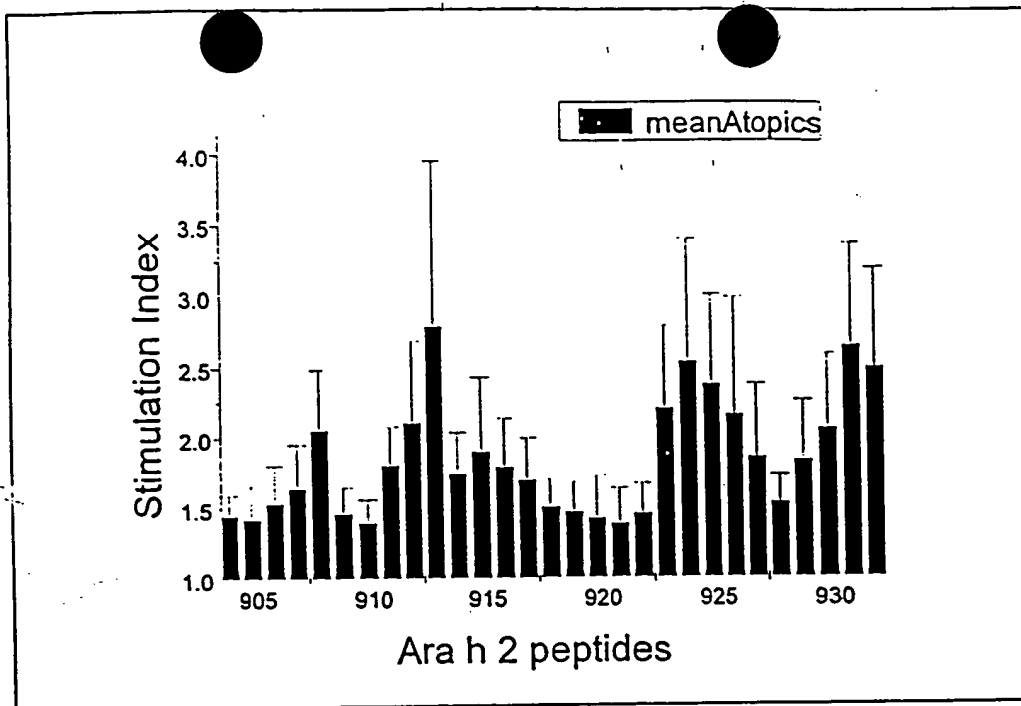
Fig. 24D



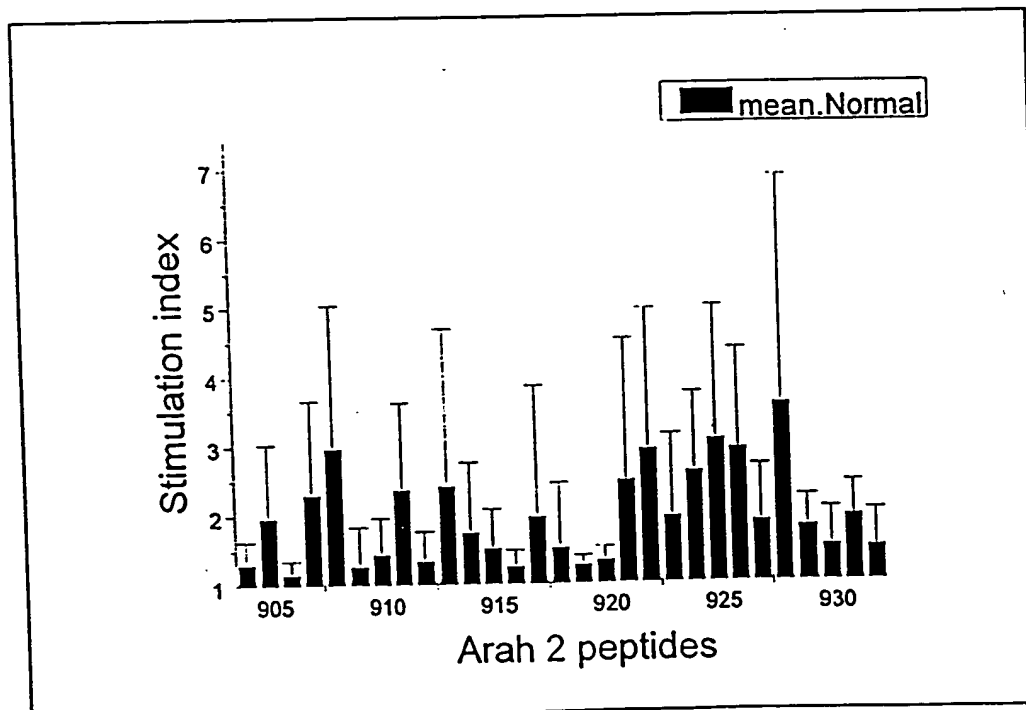
**Fig. 25 Synthetic overlapping peptides of Ara h 2.** In order to determine the T-cell epitopes of peanut allergen Ara h 2, 29 different peptides representing the entire protein were synthesized. Each peptide was 20 amino acids long and was offset from the previous peptide by 5 amino acids. In this manner we were able to cover the entire protein sequence by overlapping peptides. The primary amino acid sequence of the Ara



Panel A



Panel B

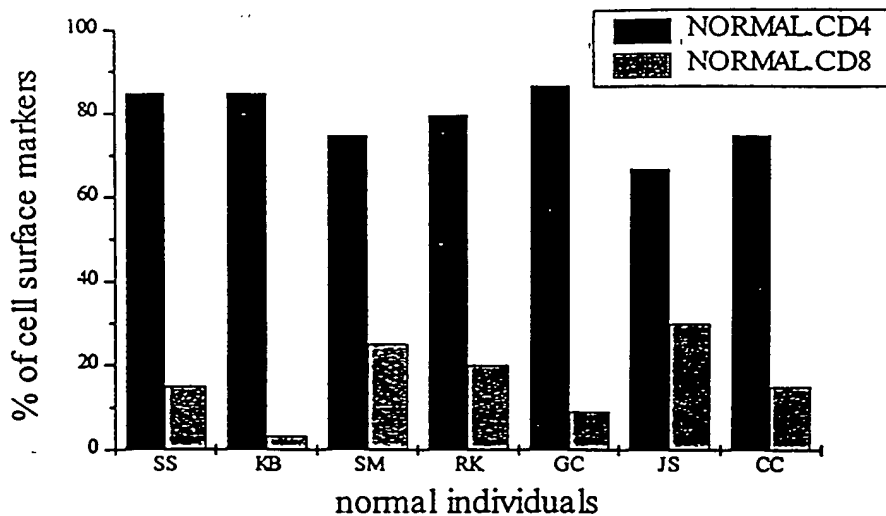


Identification of the Ara h 2 peptides that caused T-cell proliferation in the majority of patients tested.

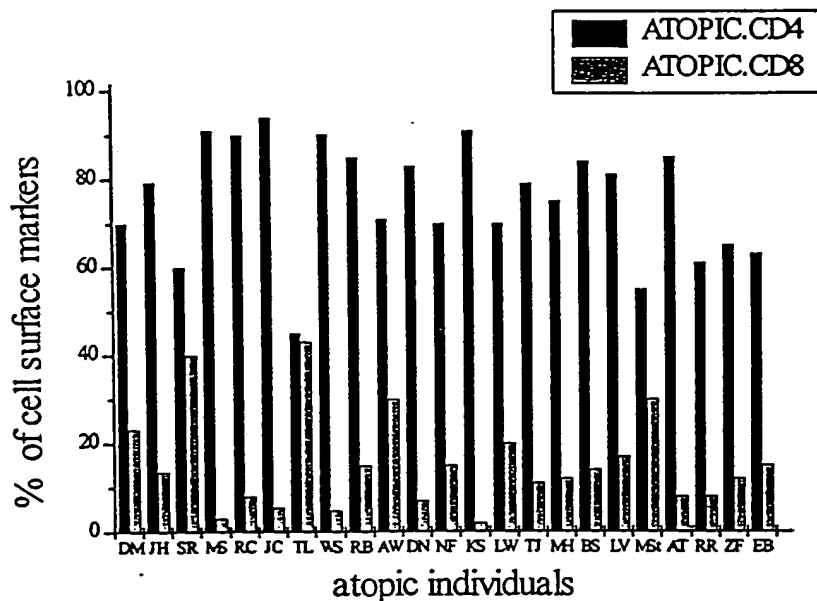
All of the data in Figure 2 was compiled and plotted as a stimulation index versus the Ara h 2 peptides. The mean proliferation and standard error of (panel A) 17 peanut allergic individuals and (panel B) 5 non-allergic individuals were calculated and plotted as mean stimulation index of atopic individuals versus the 29 overlapping peptides spanning the entire Ara h 2 protein from the amino-(peptide 904) to carboxyl terminus (peptide 932).

Fig. 27

Panel A.



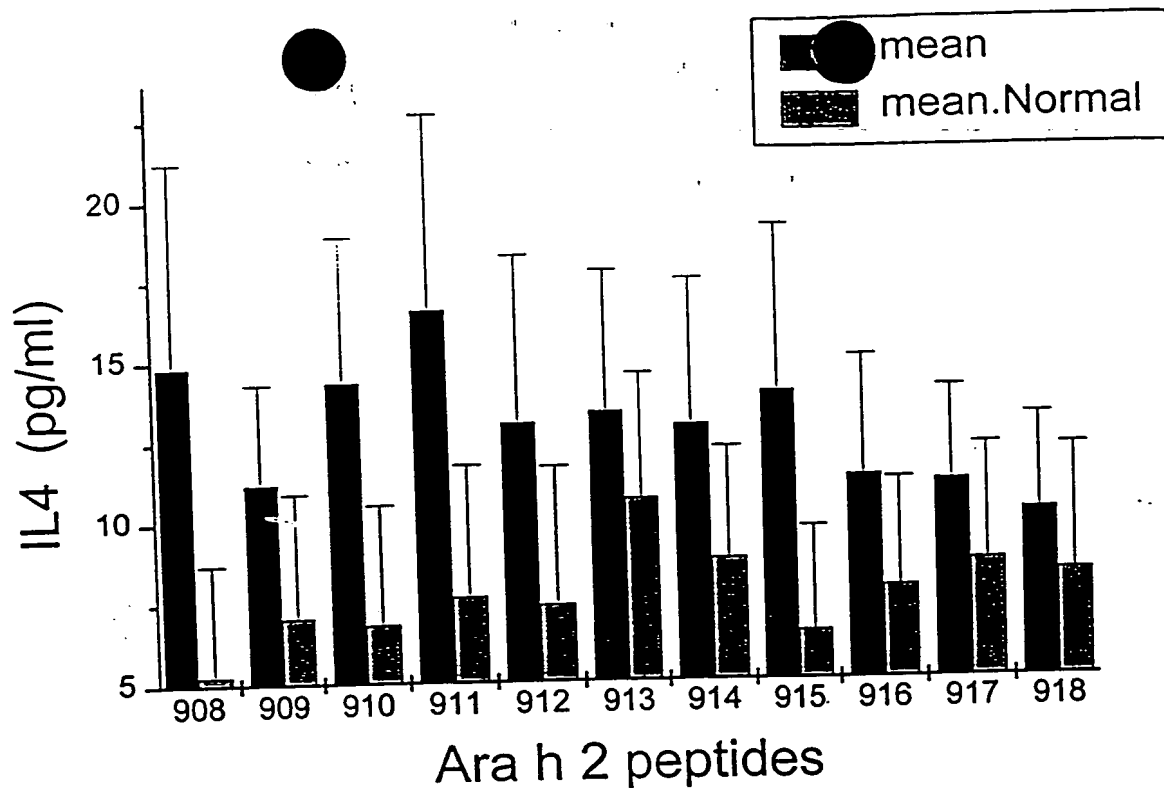
Panel B



The CD4<sup>+</sup> and CD8<sup>+</sup> profiles of the T-cell lines of peanut allergic individuals.

T cells were stained with FITC-labeled anti-CD4 and FITC-labeled anti-CD8 antibodies in order to determine the phenotype of the peanut specific T-cell lines established. FACS analysis was used to determine the percent of CD4<sup>+</sup> and CD8<sup>+</sup> cells in the peanut specific T-cell lines utilized in Ara h 2 epitope mapping and plotted versus the initials of the individual patients used to establish these cell lines. Panel A represents the CD4/CD8 profiles of T-cell lines established from allergic individuals while panel B represents the CD4/CD8 profiles of T-cell lines established from non-allergic individuals.





### The IL-4 secretion profiles of a representative sample of T cells.

The supernatant was collected from T-cells stimulated with immunodominant peptides and an ELISA assay was utilized to measure IL-4 concentrations in the media. IL-4 concentration is plotted versus the 29 overlapping peptides spanning the entire Ara h 2 protein from amino- (peptide 904) to carboxyl terminus (peptide 932).

Fig. 29

T-1

TILVALALFLLAAHASARQQWELQGDRRCQSQLERANRP

B-3

T-2

CEQHLMQKIQRDEDSYERDPYSPSQDPYSPSPYDRRAGS

B-6

B-7

T-3

SQHQERCCNELNEFENNQRRCMCEALQQIMENQSRLQGRQ

T-4

QEQQFKRELRNLPQQCGLRAPQRCDLDVESGRDY

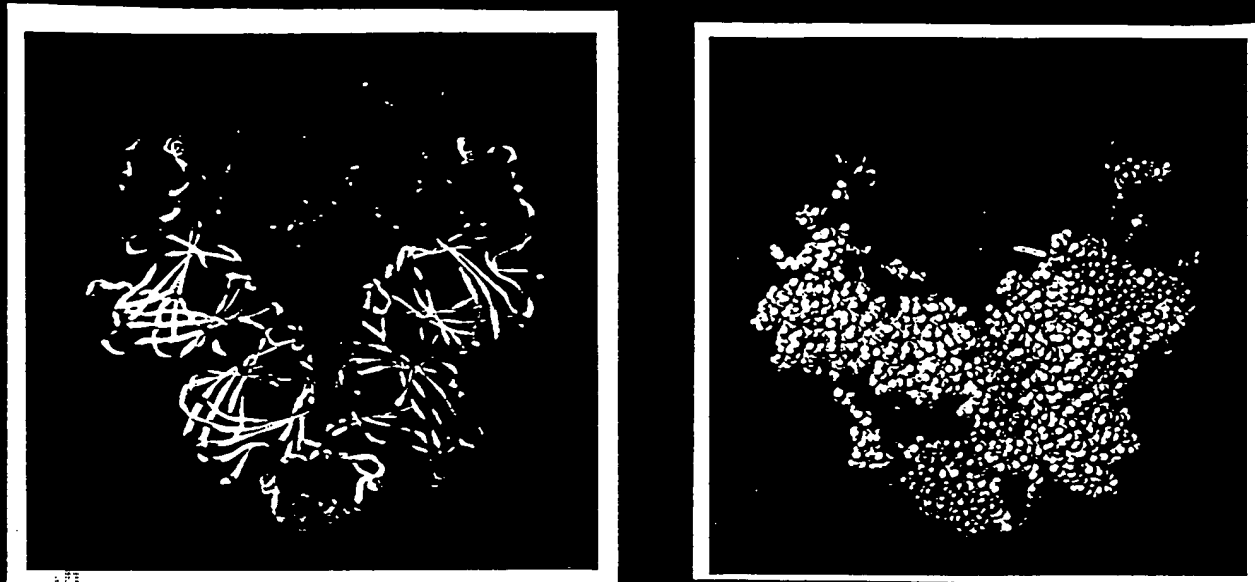
B-CELL EPITOPES

T-CELL EPITOPES

### Comparison of the T-cell and B-cell epitopes of Ara h 2.

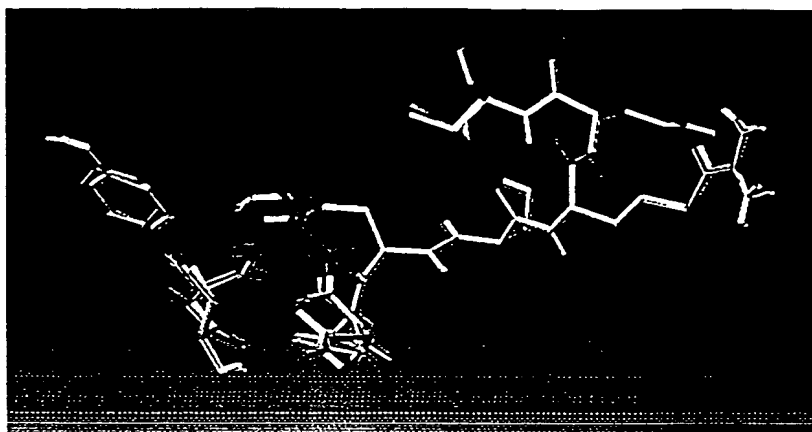
The primary amino acid sequence of the Ara h 2 protein is represented as the one letter amino acid code. The T-cell epitopes of Ara h 2 that have been identified in this study are depicted as bold, italicized letters and the immunodominant B-cell epitopes determined in previous work are underlined. In general, the IgE binding epitopes do not overlap with the T-cell epitopes.

Fig. 30



**Fig. 3| Trimeric model of the Ara h 1 molecule.**

It has been shown that other vicilin homologs form trimeric structures. Ara h 1 was also determined to form a trimeric structure by fluorescence anisotropy and cross linking experiments (see poster # 994). Ara h 1 was modeled in trimeric form by aligning the constructed model (see fig. 1 and 4) to monomers A, B and C of the trimeric template molecule phaseolin. The left panel is a ribbon diagram of the trimer with each monomer represented in a different color. The right panel is a space filled diagram where the epitope regions on the white monomer are shown in red and the epitope regions on the yellow monomer are shown in orange. This shows that a clustered epitope region on one side of a monomer joins the opposite clustered epitope region of another.



**Fig.32- Determination of residues targeted for mutagenesis in the Ara h 1 gene.**

Residues that were found to abolish IgE binding through peptide mutagenesis (see Fig. 2 and Table 1) were mutated and analyzed in the molecular model to establish which residues would less likely interfere with protein stability. The wild type form of epitope 11, **SYLQEFSRNT**, is shown in white with the leucine chosen to be mutated in red. The gold peptide is the mutated form with a methionine substitution shown in purple. The mutant was locally annealed. This substitution is predicted to leave the molecule in the least disturbed conformation and has the lowest energy compared to the other residues that were shown to abolish IgE binding when mutated (shown in bold: **SYLQEFSRNT**). The other modeled epitopes were analyzed using the same method and one or more choices per epitope appeared to be suitable for mutagenesis without altering the tertiary structure of Ara h 1 significantly.

1  
 MRGRVSPMLMLLLGILVLASVSATHAKSSPYOKKTEPCAQRCLGSCQEPDEDEKOKACHSCCTKTEYD  
 2  
 3  
 Ara h 1  
 PR  
 MMRAFP LLLGLVFLASVSFGIAY WEKENPKHNKCLQSCNSERDSYRNQACHARCNLLKVEKECEGEIAPRPR  
 β-Conglycinin  
 Region 1  
 Region 7

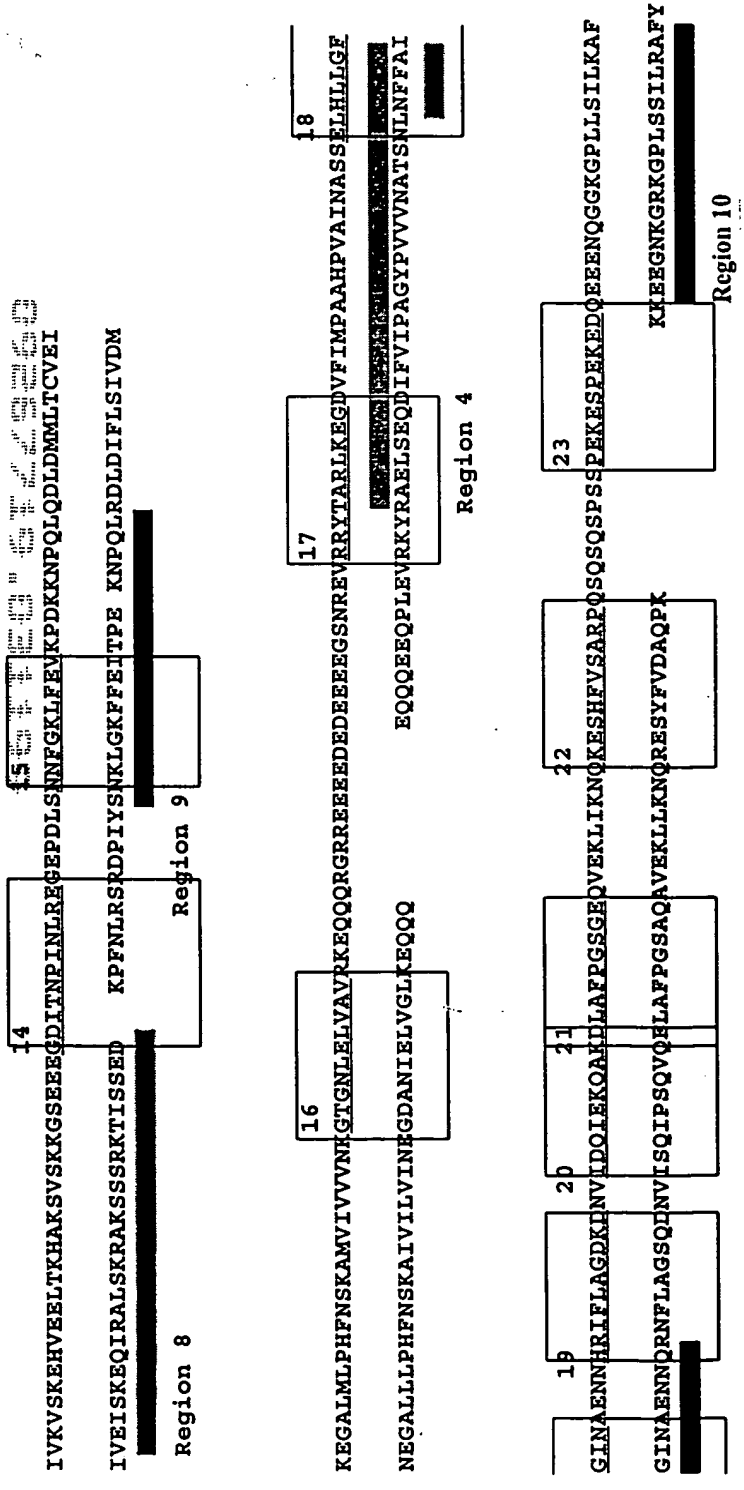
4  
 CVTPR GHTGTTNQRSPPERT RGRCPGDDYDDRRPRREGGRWG PAGPRERERE  
 5  
 6  
 7  
 EDW  
 PQHREFPQPGKEEDEDEQPRPIPPRP\*QPRCEEHEQREEQEWPRKEEKRGKSGSEEDD\*\*\*\*\*EDE  
 Region 2

8  
 RQPTED WRRPSHOOPRKIRPEGREGEQEWGTPGSHVR E ETSRNNPFYFPSPRRFSTRYGNQNGRIRVLQRFQSRQFQNLQN  
 9  
 EQDHRQFPFPPRPHQKEERNEEDEDEEQQRESESESDS\*\*\*\*ELRRHKNKNPFLFGSNRFEFLFKNQYGRIRVLQRFNQRSPQLQNLRD  
 Region 2

HRIVQIEAKPNTLVLPKHADADNLIQOQATVTVANGNNRKSFNLDGHALRIP SGFIS  
 YRLEFNSKPNTLLPNHADADYLVILNGTALTSLVNDDRDYSYRLQSGDALRVP\*\*\*\*\*SGTTY

Region 5

10  
 YILNRHDNQLRVAKISMPVNTPGOFEDFPASSRDQSSYLOGFSRNTLEAAFNAEFNEIRRVILLEENAGGEOEERGORRSTRSENNEGV  
 11  
 12  
 13  
 YVVPDNNENLRILTALPVPKPRFESFPLSSIEAQSYLOGFSRNILESAYDTKFEINKLPSREEGQOQGEQ  
 Region 3  
 Region 6



SOYBEAN SEQUENCE HOMOLOGY TO ARA H 1  
 SOYBEAN AND PEANUT POSITIVE REGIONS  
 15-MERS X 8 SPOTS ANALYSIS

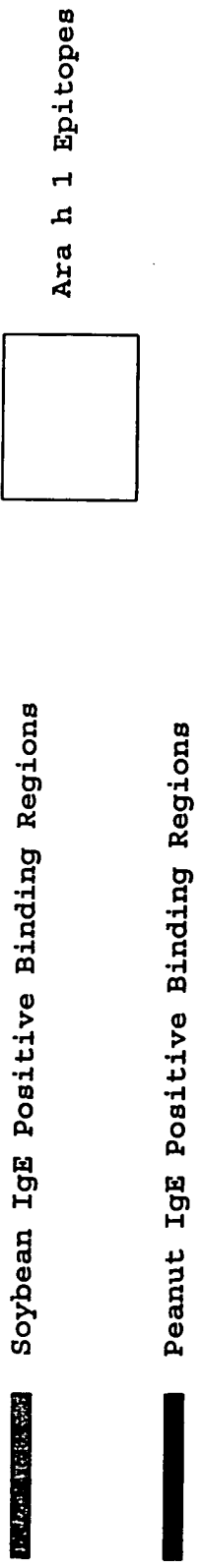


Fig. 33B

# SEQUENCE HOMOLOGY OF ARABINOSYL-BINDING EPITOPES IN REGIONS OF BETA CONGLYCININ

EPITOPE 1 AKSSPYQKKT GIAY WEK	EPITOPE 2 QEPDDLKQKA SERDSYRNQA * * *	EPITOPE 3 LEYD LKVEKEECEGEIIPRRPRQHP * **	EPITOPE 4 GERTRGRQPG FPRQPQRQEE * **	EPITOPE 5 PSDYDDDRRQ EEEHEQREEQ *
EPITOPE 6 PRREGGRWG EWPRKEEKG *	EPITOPE 7 REREEDWRQP EDEDEDEDEQ *	EPITOPE 8 EDW RRPSSHQQ RQFPFPRPPHOK ** **	EPITOPE 9 QPRKIRPEGR KEERNEEDE *	EPITOPE 10 TPGQFEDFFP KPGRFESFFL ** **
EPITOPE 11 SYLQEFNRNT SYLQGFNRNI ****	EPITOPE 12 FNAEFNEIRR YDTKEEINKV	EPITOPE 13 EQEERGQRRW QQGEQRILQE * *	EPITOPE 14 DITNPINLRE KPFNLRS * ***	EPITOPE 15 NNFGKLEVK NKLKGFTEIT * **
EPITOPE 16 GTGNLELVAV GDANIELVGL * * *	EPITOPE 17 RRYTARLKEG RKYREELSEQ * * *	EPITOPE 18 ELHLLGFGIN NLNFFAIGIN * ***	EPITOPE 19 JRIFLAGDKD QRNFLAGSQD **** *	EPITOPE 20 IDQIEKQAKD ISQIPSQVQE * **
EPITOPE 21 KDLAFPGSGE QELAFPGSAQ *****	EPITOPE 22 KESKFVSARP RESYFVDAQP ** ** *	EPITOPE 23 PEKESPEKED K		

Fig. 34

## cDNA CLONING

Soybean seeds, *Glycinus max*, Hutchinson variety, were obtained from a local health food store, frozen in liquid nitrogen, ground to a fine powder, and the RNA extracted using the method of Nedergaard et al (Mol Immunol 29:703,1992). Briefly, 2 g frozen seed powder was added to 10 mls buffer (250 mM sucrose, 200 mM Tris-HCl, pH 8.0, 200 mM KCl, 30 mM MgCl<sub>2</sub>, 2% polyvinylpyrrolidone-40 and 5 mM 2-mercaptoethanol) and equilibrated with 10 ml fresh phenol (4°C). The suspension was homogenized and 10 ml of chloroform added with shaking for 5 min at RT. Phases were separated by centrifugation, 10k g for 20 min at 4°C and the aqueous phase transferred to a clean test tube and extracted 2x with equal volumes of chloroform/phenol. Nucleic acids were precipitated with sodium acetate/ethanol at -20°C overnight. The precipitates were collected by centrifugation at 13k g for 20 min at 4°C, washed with 70% ethanol and dried. Samples run in parallel were pooled in water and made 3M in LiCl, and the RNA precipitated for 4 hr at -20°C. The precipitate was collected by centrifugation outlined above and resuspended in distilled water. Fifty microliters of the RNA suspension was withdrawn for OD<sub>260/280</sub> measurements and the RNA analyzed by agarose gel electrophoresis. Three aliquots representing a total of approximately 3.0 mgs total RNA was sent to STRATAGENE for purification of mRNA and the preparation of a Uni-Zap XR custom library.

The expression custom library was screened with serum from soybean-sensitive individuals and positive clones subcloned to homogeneity with respect to IgE-binding. Five clones were isolated from an initial screen and the plasmids purified from LB/ampicillin broth cultures using an Amersco kit. The plasmid DNA from each clone was PCR amplified and analyzed in agarose gels. Two plasmid preparations had relative bp of approximately 1400 and the remaining three 1500 bp.

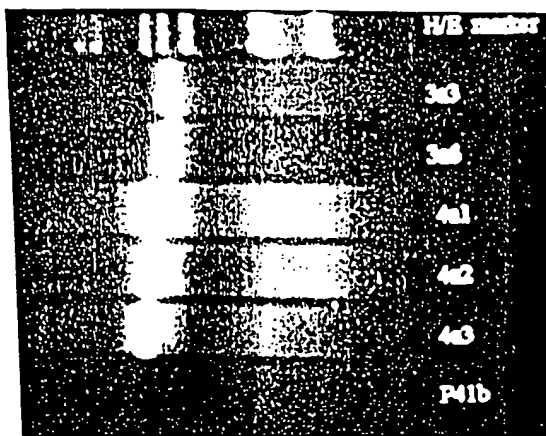


Fig. 35

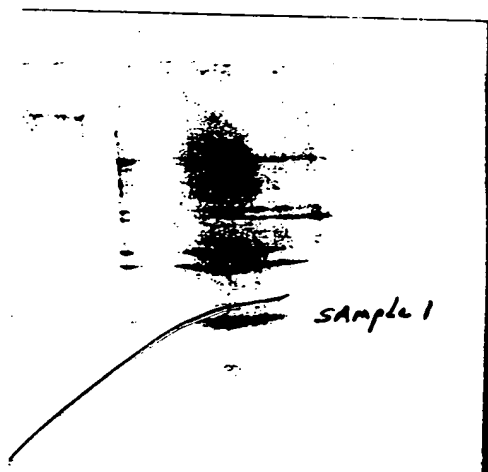
## PCR AMPLIFIED PLASMIDS ISOLATED FROM SOYBEAN CDNA EXPRESSION LIBRARY



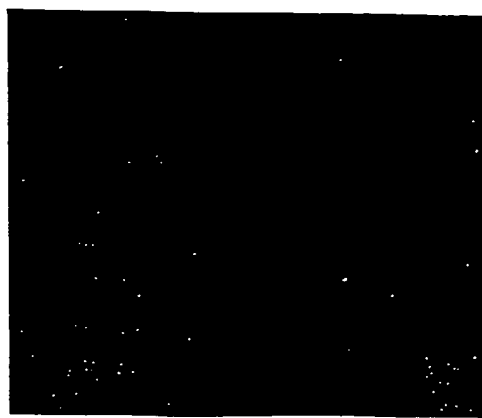
## ALLERGEN IDENTIFICATION BY 2-D SDS-PAGE

A crude soybean extract was applied to a 12.5% preparative SDS-PAGE gel and electrophoresed using a BIO-RAD prep cell. Five ml fractions were collected and aliquots were electrophoresed into a Pharmacia 24-well 10% horizontal gel, electrophoretically transferred to a nitrocellulose membrane, the remaining sites blocked using PBS/0.05% Tween 20, and analyzed for IgE-binding using serum from soybean-sensitive individuals. Fractions that bound IgE were dialyzed against 100mM ammonium bicarbonate (x4 x 4 liters) for 24 hours, lyophilized, reconstituted in distilled water and analyzed by 2-D (isoelectric focusing in the first dimension, pH 3-7, followed by a 4-20% SDS-PAGE gel molecular weight separation in the second) in duplicate. The proteins in the duplicate gels were transferred to nitrocellulose membranes, one was stained with Coomassie blue for protein identification and the other was prepared for IgE immunoblot analysis. IgE-binding proteins were identified by radiolabeled anti-IgE and X-ray autoradiography. Positive IgE-binding proteins by autoradiography were compared to the Coomassie stained gel protein profile. The stained blot was submitted to the Yale Biotechnology Center for amino acid sequencing. The results of this analysis revealed a 20-22kD protein with significant homology to the A2B1a glycinin protein family. Additional samples are being assessed for activity and identification.

### A: Coomassie blue stained 2-D SDS-PAGE gel



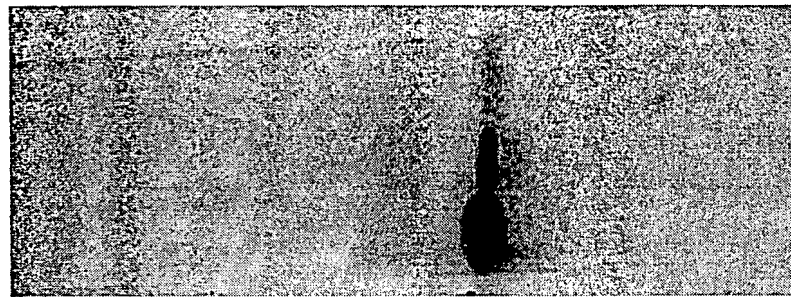
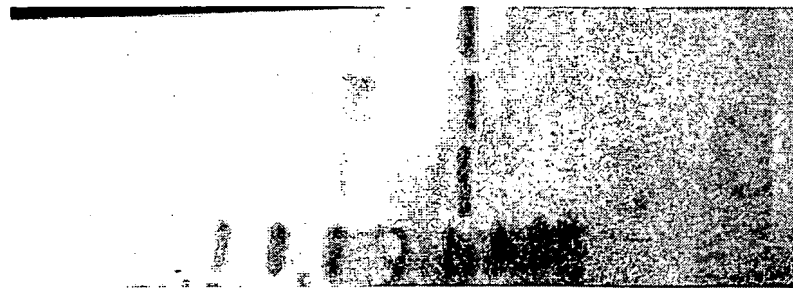
### B: IgE immunoblot of 2-D SDS-PAGE blot



# IgE BINDING OF rAra h 2 PROTEINS IN WESTERN BLOT ANALYSIS

T7 tag

Serum IgE



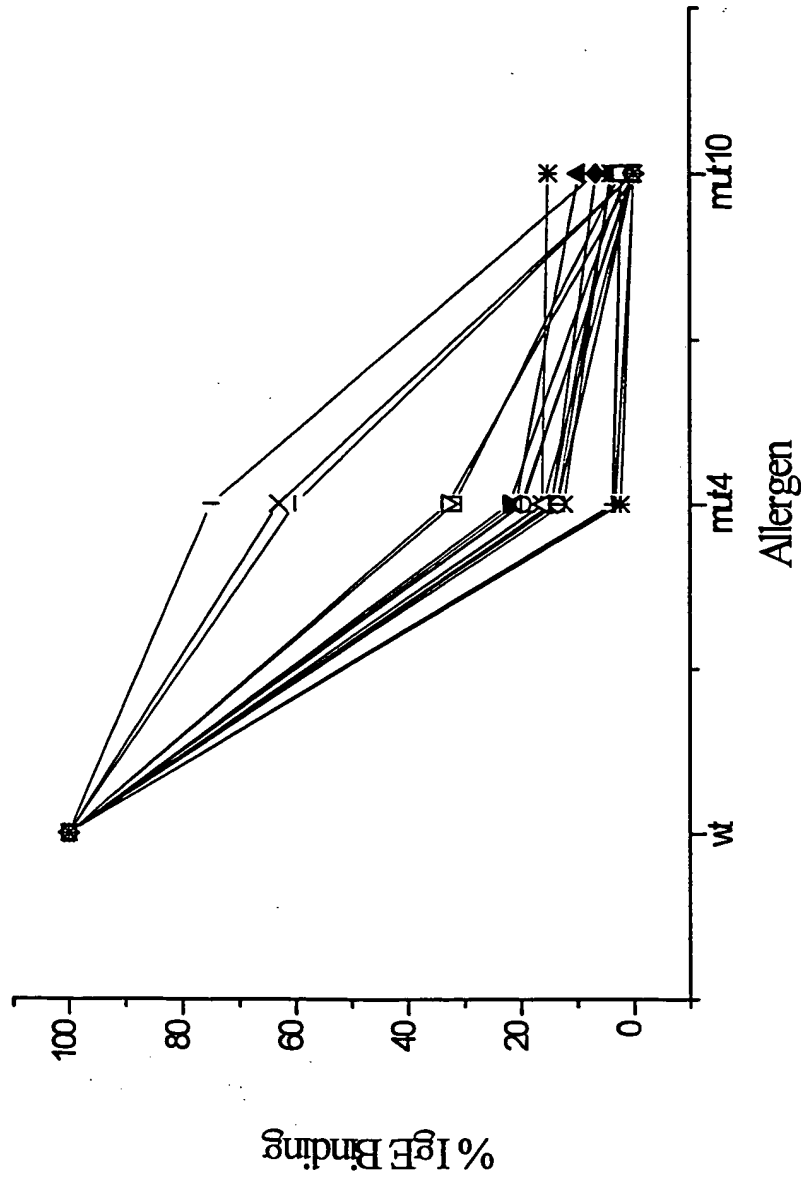
MW 10 6 0

10 6 0

Number of epitopes

100 80 60 40 20 0

**Figure 37 B**  
**SERUM IgE BINDING OF rAra h 2 PROTEINS**  
**IN INDIVIDUAL PATIENTS**



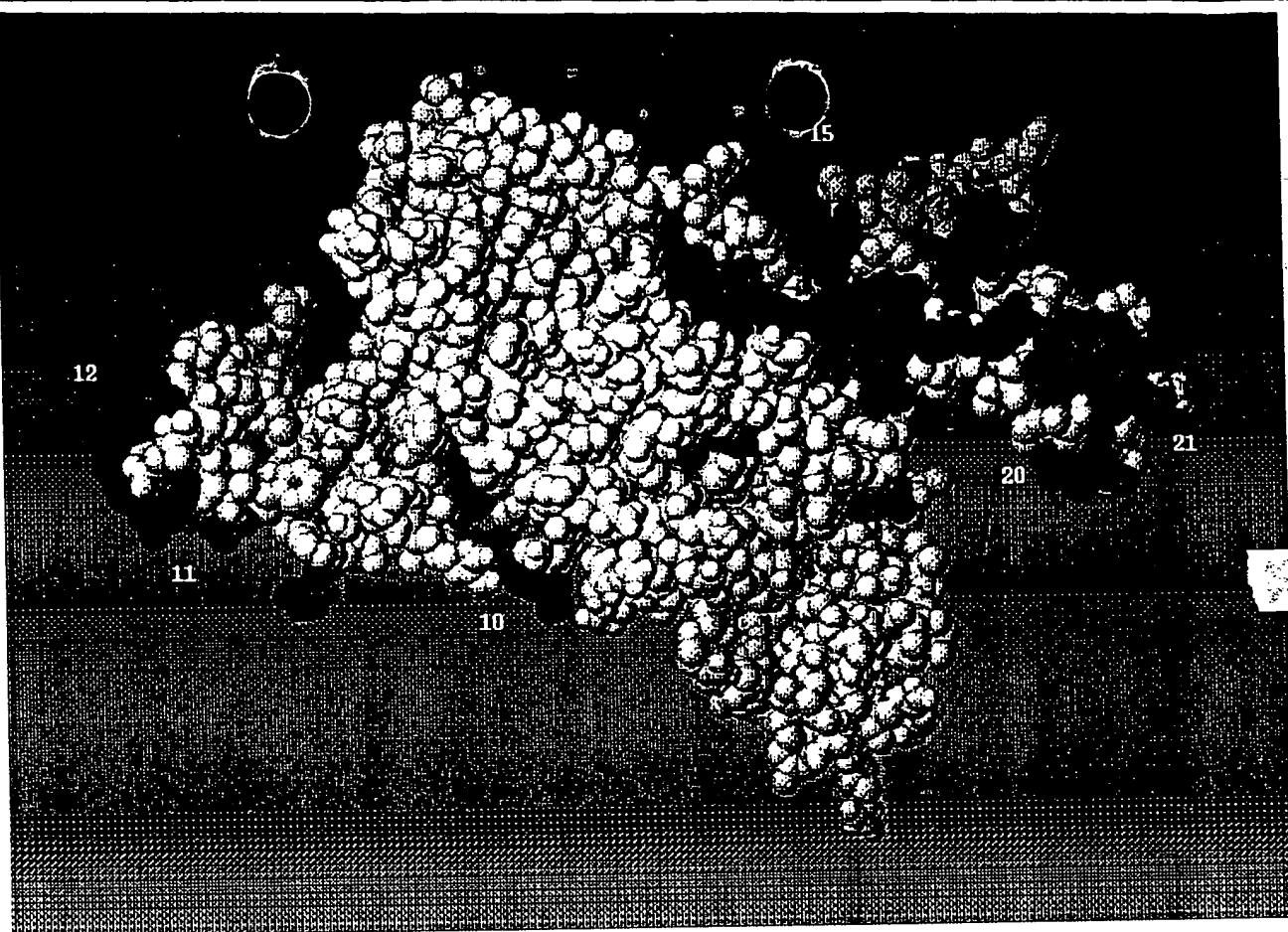


Fig. 38 Upper Panel

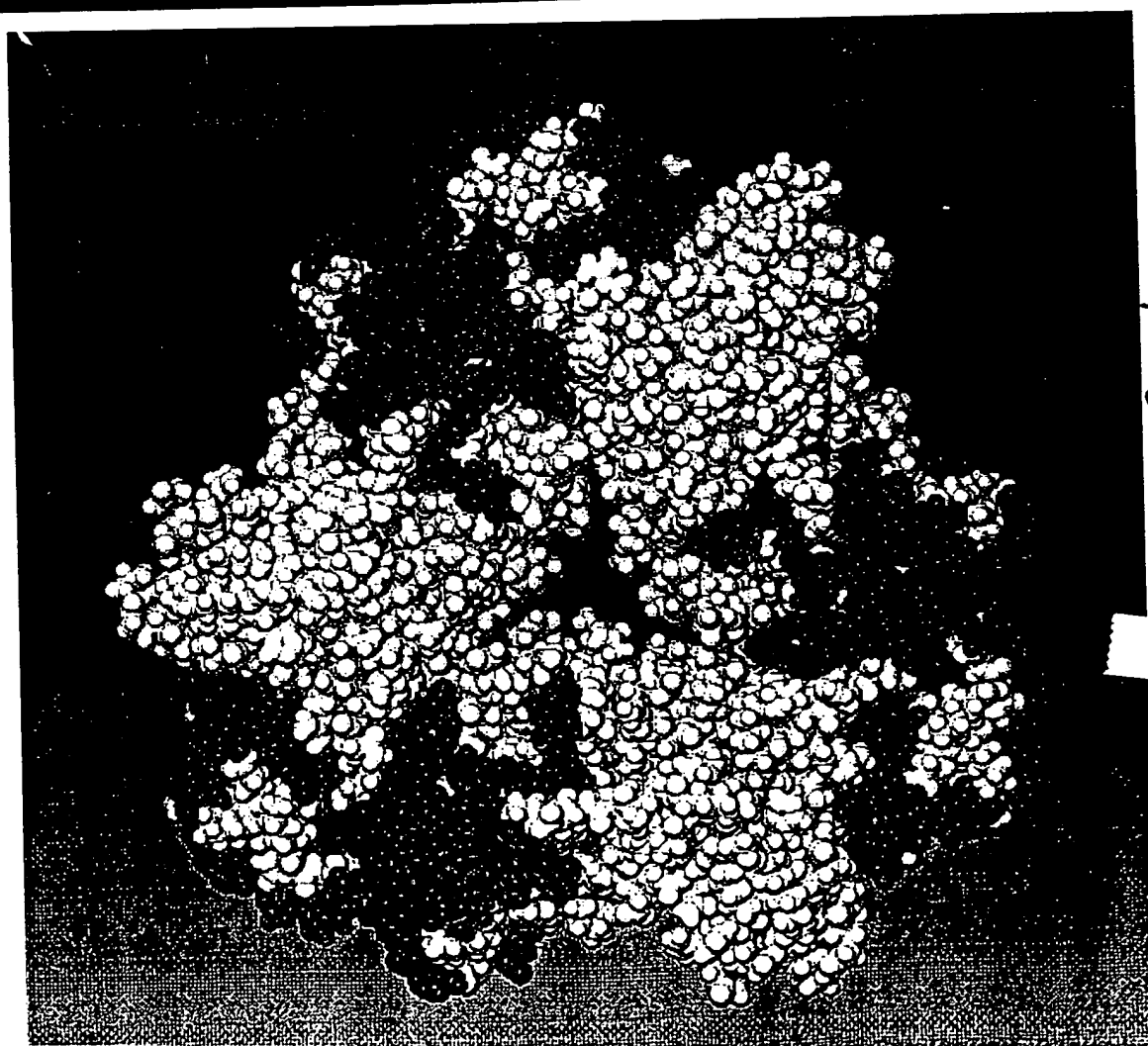


Fig. 38 Lower Panel

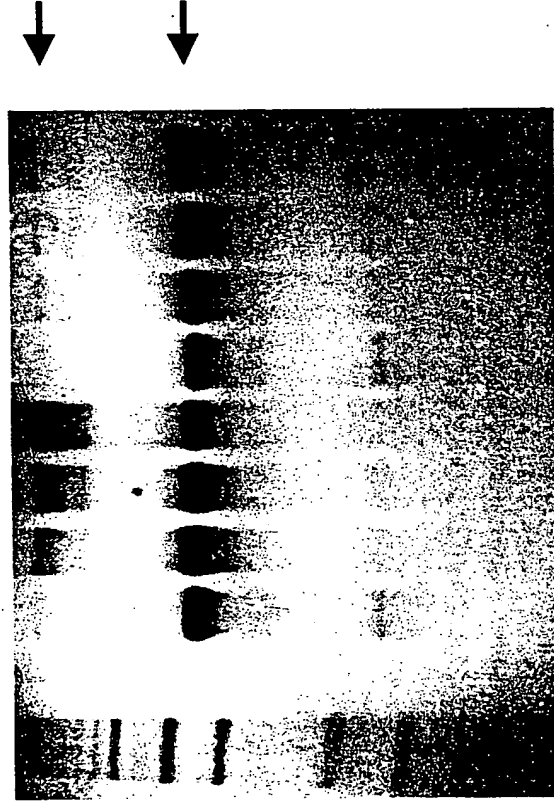
## Ara h 1- Pepsin/Chymotrypsin Sites

MRGRVSPMLLLGILVLASVSATHAKSSPYQKKTENPCAQRCLQSCQQEP  
DDLKQKACEESRCTKLEYDPRCVYDPRGHTGTTNQRSPGERTGRQPGD  
YDDRRQPRREEGGRWGPAGPREREREEDWRQPREDRRPSHQPRKIRP  
EGREGEQEWGTPGSHVREETSRNNPFYFPSRRFSTRYGNQNGRIRVLQRFD  
QRSRQFQNLQNHRIVQIEAKPNTLVLPKHADADNILVIQQGQATVTVANGN  
NRKSFNLDEGHALRIPSGFISYILNRHDNQNLRAAKISMPVNTPGQFEDFFFA  
SSRDQSSYLQGFSRNTLEAAFNAEFNEIRRVLLEENAGGEQEEERGQRRWST  
RSSENNEGVIVKVSKEHVEELTKHAKSVSKKGSEEEGDITNPINLRHGEPDLS  
NNFGKLFVVKPDKKNPQLQDLDMMLTCVEIKEGALMLPHFNSKAMVIVVV  
NKGTGNLELVAVRKEQQQQRGRREEEDEDDEEEEGSNREVRRTARLKEGD  
VFIMPA AHPVAINASSEHLLGFGINAEENNHRIFLAGDKDNVIDQIEKQAKD  
LAFPGSGHQVEKLIKNOKESHFVSARPOQSQSPSSPEKESPEKEDQEEENQG  
GKGPLL SILKAFN

# Ara h 1- Trypsin Sites

MRGRVSPMLLLGILVLASVSATHAKSSPYQKKTENPCAQRCLQSCQQEP  
 DDLKQKACEESRCTKLEYDPRCVYDPRGHTGTNQRSPGGERTRGRQPGD  
 YDDRRQPRREEGRWGPAGPREREREEDWRQPREDDWRRPSHQQPRKIRP  
 EGRJEGEQEWGTPGSHVREETSRNNPFYFPSRRFSTRYGNQNGRIRVLQRFD  
 QRSRQFQNLQNHRIVQIEAKPNTLVLPKHADADNILVIQQGQATVTVANGN  
 NRKSFNLDEGHALRIPSGFISYILNRHDNQNLRAAKISMPVNTPGQFEDFFPA  
 SSRDQSSYLQGFSRNTLEAAFNAEFNEIRRVLLEENAGGEQEEERGQRRWST  
 RSSENNEGVIVKVSKEHVEELTKHAKSVSKKGSEEEGDITNPINLREGEPDLS  
 NNFGKLFVVKPDKKNPQLQDLDMMLTCVEIKEGALMLPHFNSKAMVIVVV  
 NKGTGNLELVAVRKEQQQQRGRREEEDEDDEEEEGSNREVRRTARLKEGD  
 VFIMPAAHPVAINASSSELHLLGFGINAEENNHRIFLAGDKDNVIDQIEKQAKD  
 LAFPGSQHVEKLIKNOKESHFVSARPOQSQSQSPSSPEKESPEKEDQEEENQG  
 GKGPLLSILKAFN

M 0 10 20 50 0 10 20 50  
65 40 30 20 10 5 0

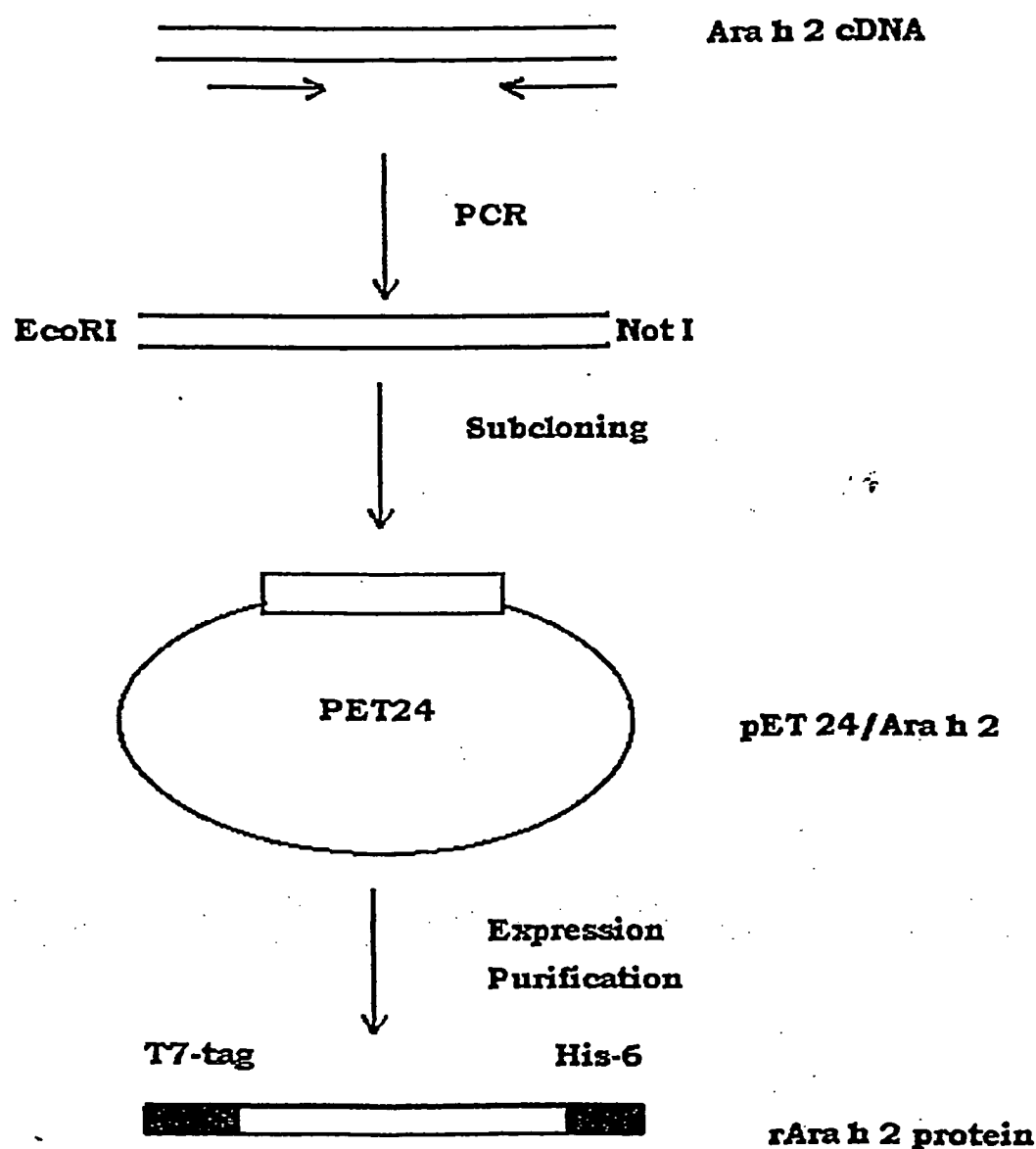


**Figure 40 The Ara h 1 trimer is unstable at pH2.**

In order to assess the stability of the Ara h 1 trimer at pHs that would be encountered in the human stomach, cross-linking experiments were performed using purified Ara h 1 protein suspended in a pH 2.1 buffer. Purified Ara h 1 (2 mM) was suspended in 500  $\mu$ l of either a pH 2.1 buffer or a pH 7.6 buffer and allowed to incubate for one hour at room temperature. Cross-linking was performed using 5% DSP in DMF for varying lengths of time (10, 20, or 50 seconds). Results indicate that the Ara h 1 trimer is unstable at acidic pHs that would be encountered in the human stomach but that the monomer is stable at this pH. Further experiments indicate that the monomer is stable at pH 2.1 for greater than 8 hours at 37°C.

# Figure 4. A

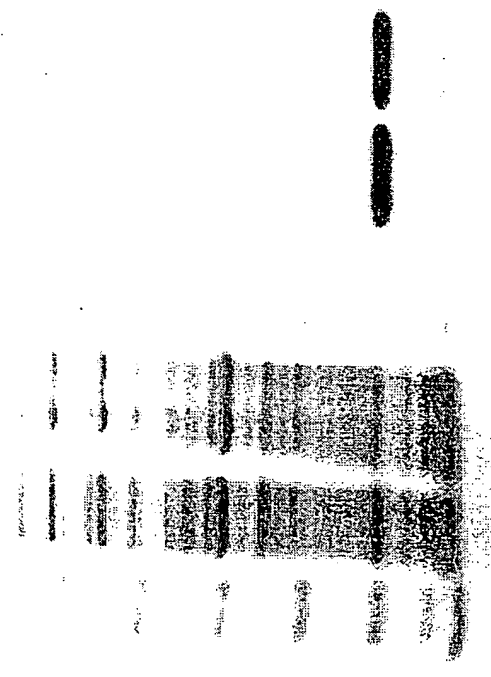
## EXPRESSION OF RECOMBINANT Ara h 2 PROTEIN IN *E. Coli*





**Figure 4/ B**

**PURIFICATION OF RECOMBINANT Ara h 2  
PROTEINS ON A Ni-COLUMN**

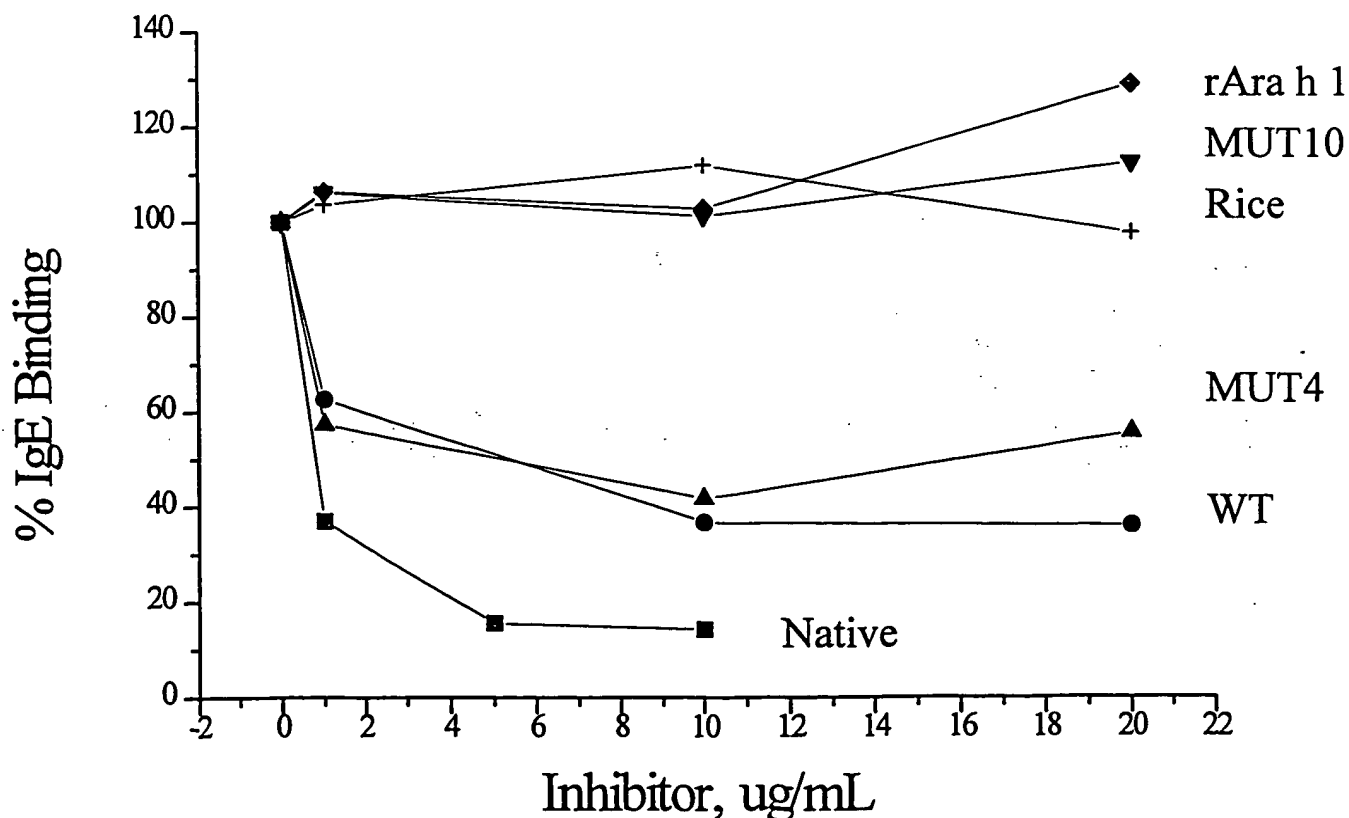


MW 1 2 3 4 5 6

# Figure 42

## INHIBITION OF IgE BINDING TO NATIVE Ara h 2 PROTEIN

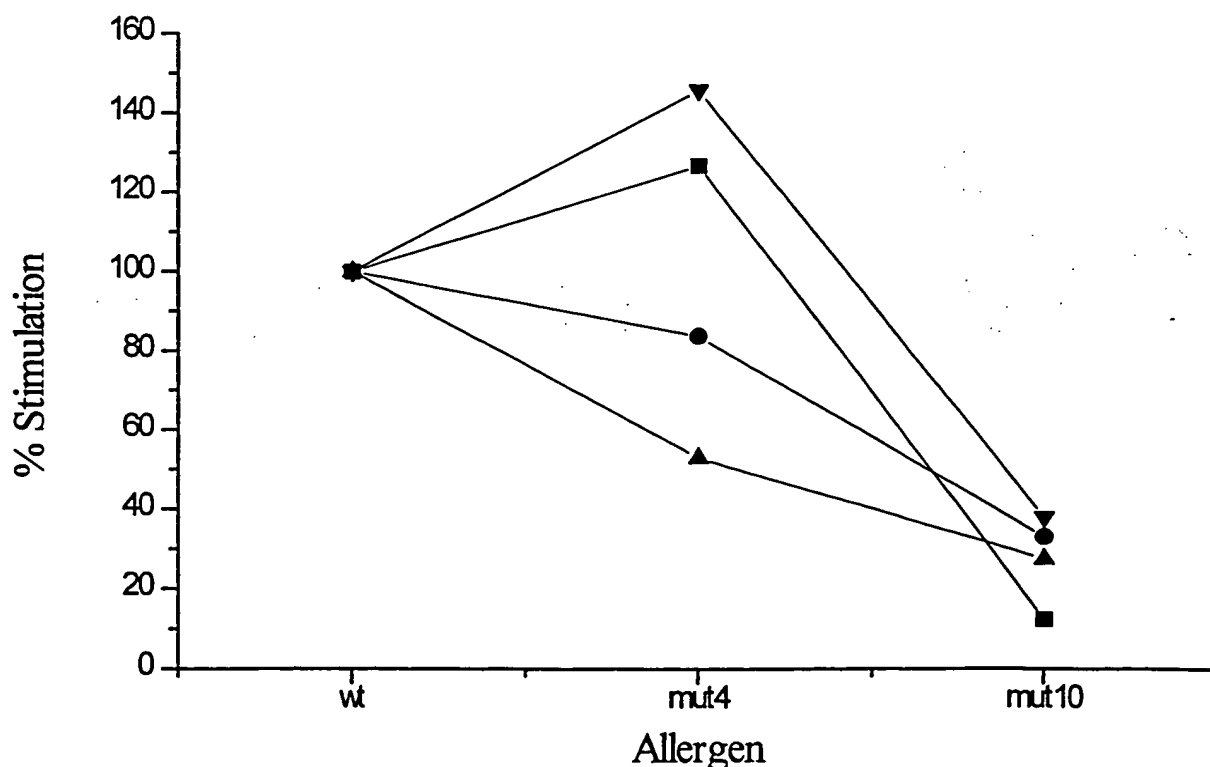
0.5 ug of the native Ara h 2 protein purified from crude peanut extracts were loaded onto nitrocellulose membrane using a slot-blot apparatus. Membranes were incubated with patient serum pool (1:20) in the presence or absence of different concentrations of wild type or mutated recombinant Ara h 2 proteins. Membranes were probed for the bound IgE with  $^{125}\text{I}$  anti-human IgE antibody. Laser densitometry of the autoradiograms was used to quantitate the relative amounts of IgE binding.

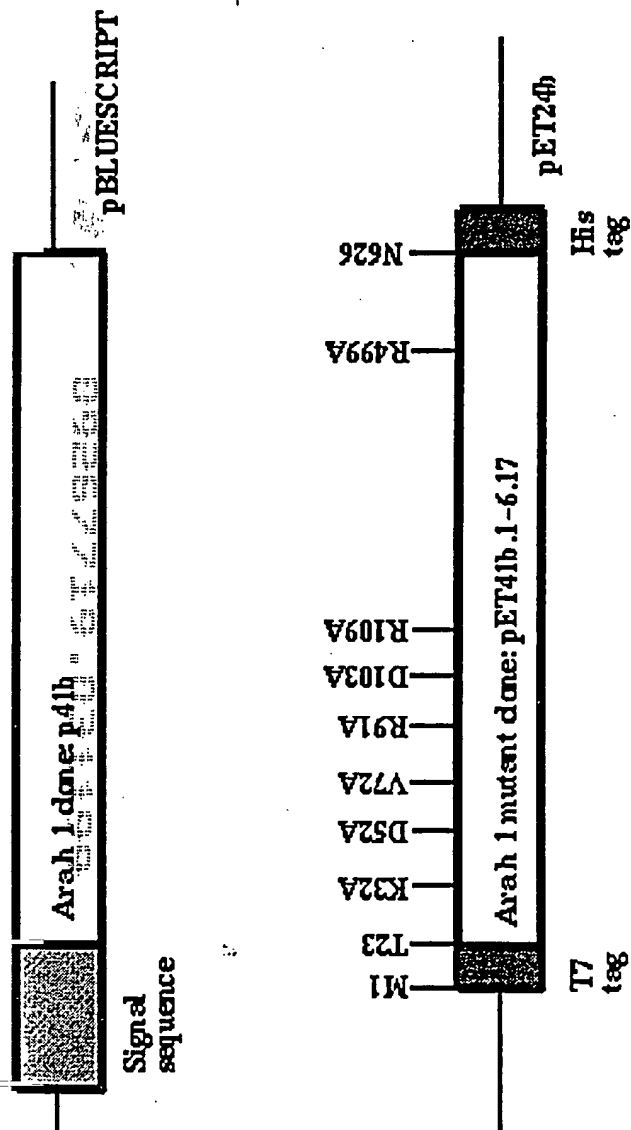


# Figure 43

## PROLIFERATION OF PBMCs FROM PEANUT SENSITIVE PATIENTS

PBMCs were isolated from heparinized venous blood of peanut-sensitive patients by density gradient centrifugation on Ficoll.  $2 \times 10^5$  cells per well were incubated in triplicates for 7 days in RPMI media with 5% human AB serum in the presence of 10 ug/ml of the native Ara h 2 protein purified from the crude peanut extract or recombinant Ara h 2 proteins purified from *E.coli*. Cells incubated in media only were used as a control. Proliferation was measured by the incorporation of tritiated thymidine. Stimulation index is calculated as a ratio of radioactivity for the cells growing in the presence of allergen to that for the cells growing in media alone.





<u>Epitope</u>	<u>Mutation</u>
1	K32A
2	D52A
3	V72A
4	R91A
5	D103A
6	R109A
17	R499A

Figure 44 Wild type and mutant Ara h 1 plasmid constructs.

The top diagram represents the original Ara h 1 p41b construct. It consists of the Ara h 1 cDNA insert within the pBLUESCRIPT SK- vector. Noted are the Met1, Thr23, and Asn626 positions. The residues Met1-Thr23 represent the Ara h 1 signal sequence and Asn626 is the C-terminal residue. The lower diagram represents the mutant Ara h 1 construct pET41b.1-6.17. It was constructed by inserting a PCR product into the pET24b expression vector. Noted are the epitope mutation positions (not drawn to scale) and the positions of the T7 and His tags. The particular mutations within each epitope are listed under the diagrams.

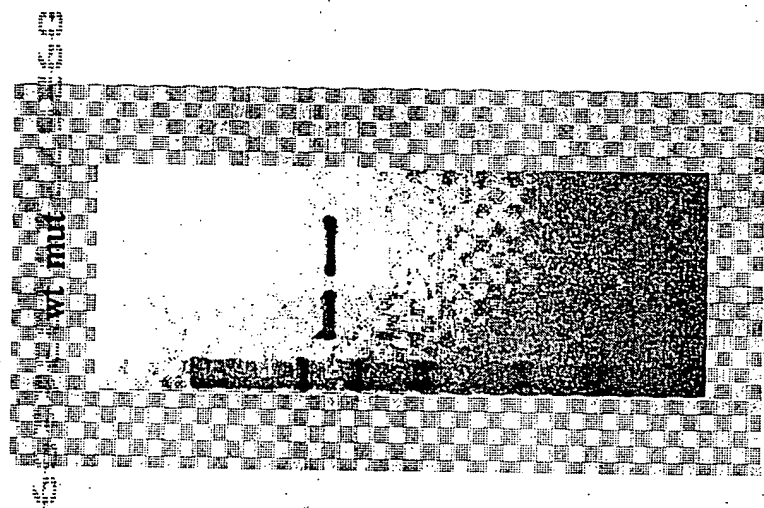
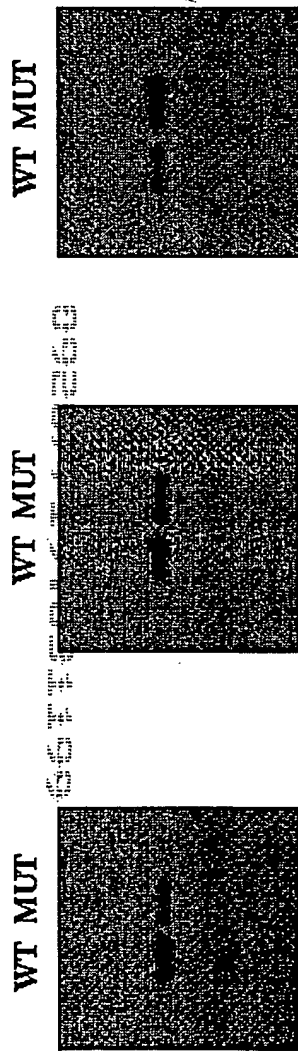


Figure 45 Wild type and mutant protein western blot control.

A western blot control was performed on the wild type and mutant Ara h 1 recombinant proteins to ensure that an equal amount of each protein was used in these studies. Novex see-blue molecular weight markers were visualized by Coomassie staining in the first lane. Equal amounts of wild type (middle lane) and mutant (right lane) were detected by probing with anti-T7 antibody alkaline phosphatase conjugate. Both proteins migrate at their expected molecular weights (65 kDa).



### Patient 1

Mutant epitopes:  
1, 4, 5, 17

WT epitopes:  
8, 13

### Patient 2

Mutant epitopes:  
2, 3, 4, 17

WT epitopes:  
14, 18

### Patient 3

Mutant epitopes:  
4, 5, 17

WT epitopes:  
11, 14, 18, 19, 20, 22

Figure 46 Mutation of the Ara h 1 protein leads to altered IgE binding.

Three western blots of wild type (left lane) and mutant (right lane) recombinant proteins probed with individual peanut-sensitive patient sera are shown. The epitopes that each patient recognized are indicated below each blot. Mutant epitopes corresponds to the epitopes that the patient recognized that were altered in the mutant protein. WT epitopes corresponds to epitopes that were recognized by the patient, but were not changed in the mutant protein. In the first panel IgE binding was decreased. In the second panel binding was roughly equal. In the third panel binding was increased.



Figure 47 Tertiary structure model of the Ara h 1 protein.

A space-filled model of the middle and C-terminal domains of the Ara h 1 allergen is shown. The red areas represent the IgE binding epitopes. The yellow atoms represent residues that were determined to be critical for IgE binding to occur. The numbers correspond to some of the epitopes listed in Table 1.

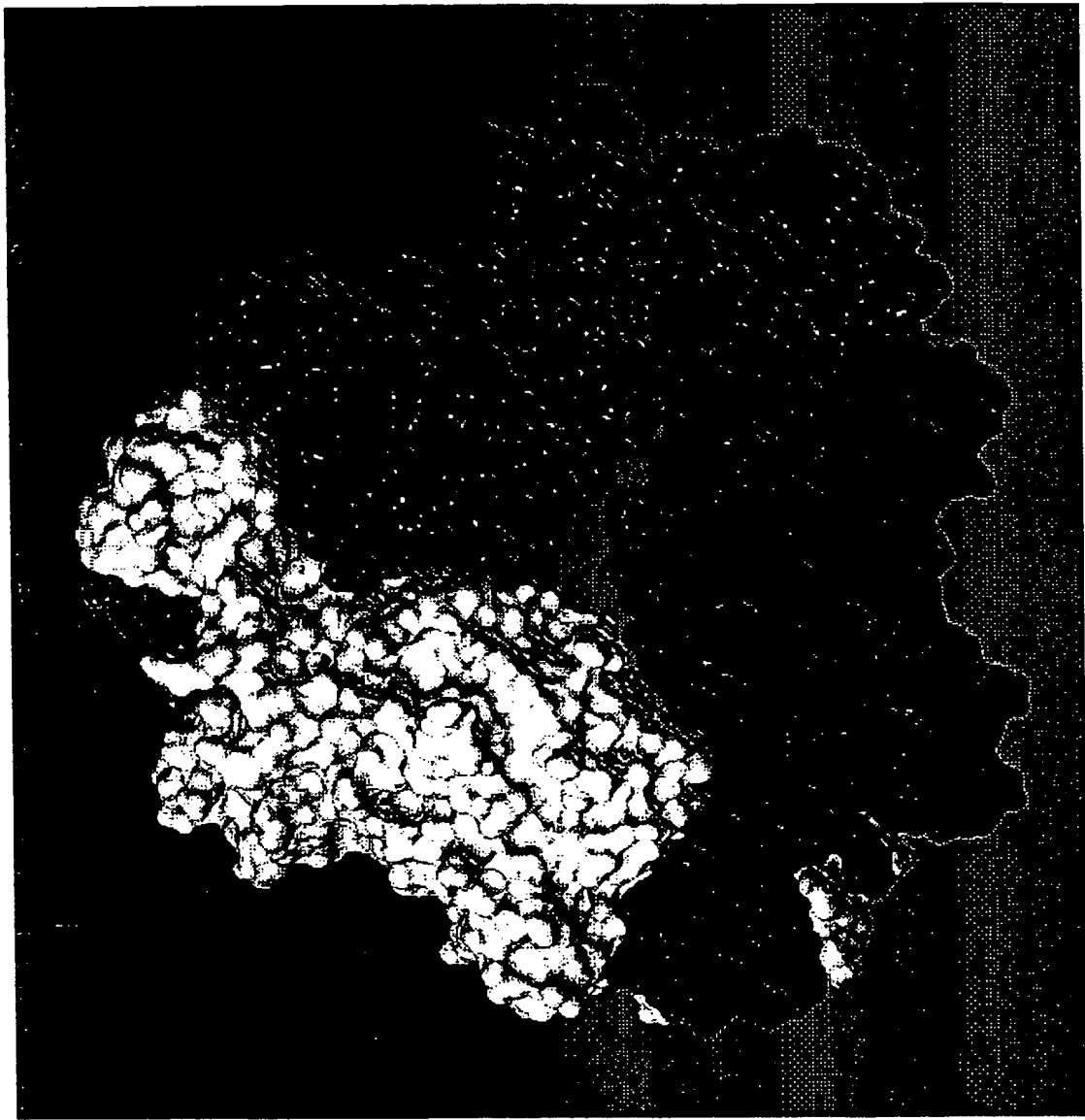


Figure 48 Contoured surface diagram of the trimeric Ara h 1 model.

A contoured surface of each Ara h 1 monomer is shown in a different color. The trimeric structure is based on the phaseolin trimer structure. The alpha helical bundles and adjacent beta sheets form the interface of monomer-monomer contact.



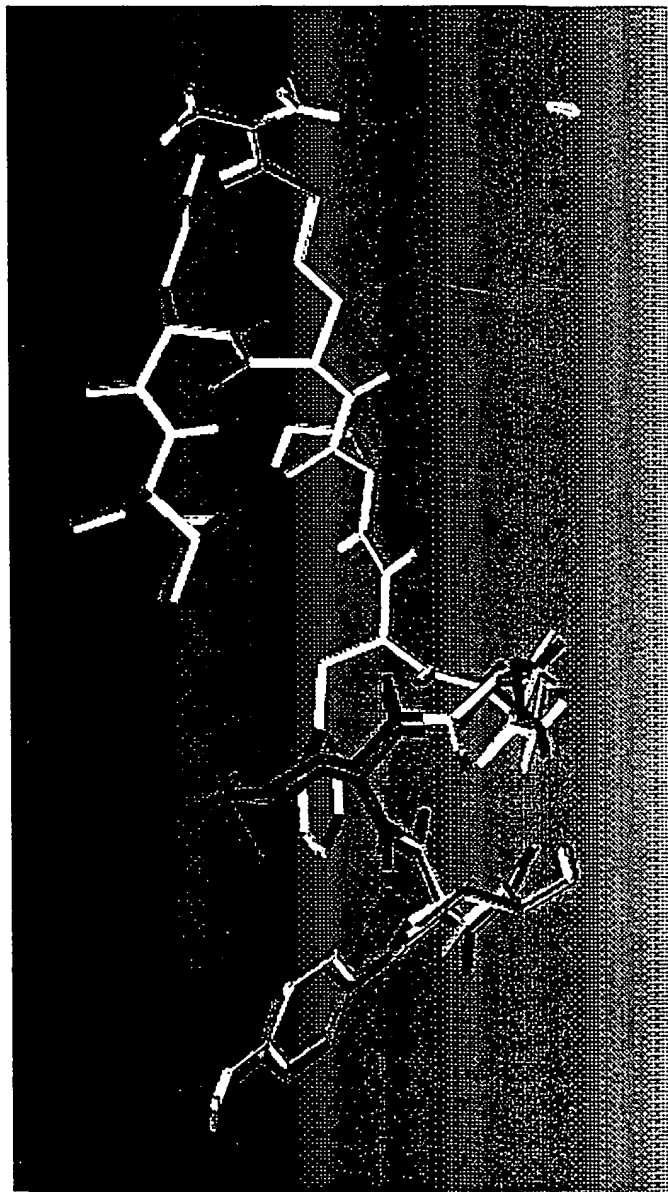
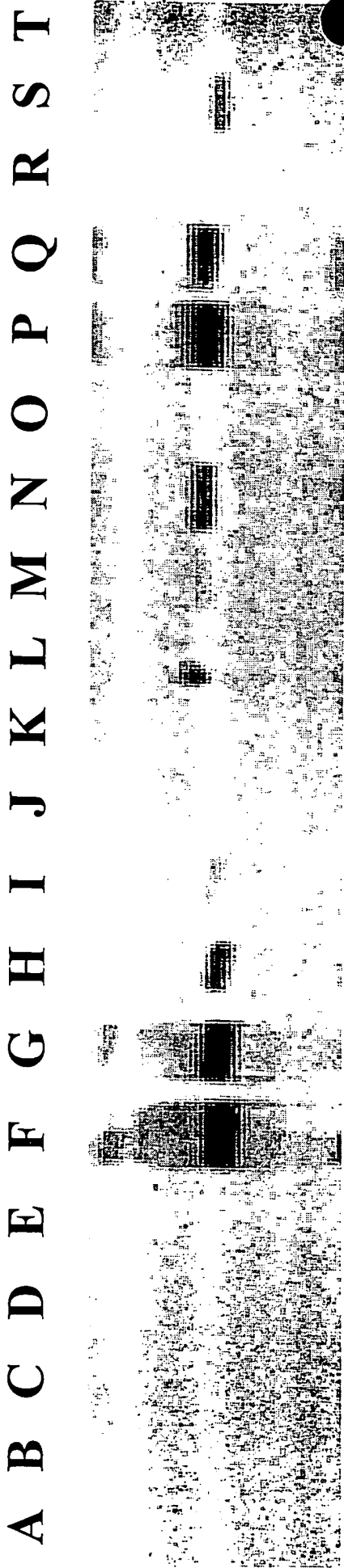


Figure 49 Calculated effect of L313M mutation on epitope 11.

A stick diagram of epitope 11 (SYLQGFSRNT) is shown in yellow with the exception of Leu 313 which is shown in red. A mutant, L313M, Ara h 1 protein was computationally generated. The region corresponding to the mutant epitope 11 region is shown in white where the substituted residue (methionine) is shown in purple. The remaining atoms of both the wild type and mutant peptide are not shown for clarity. This mutation is not predicted to alter the structure of the mutant protein substantially.

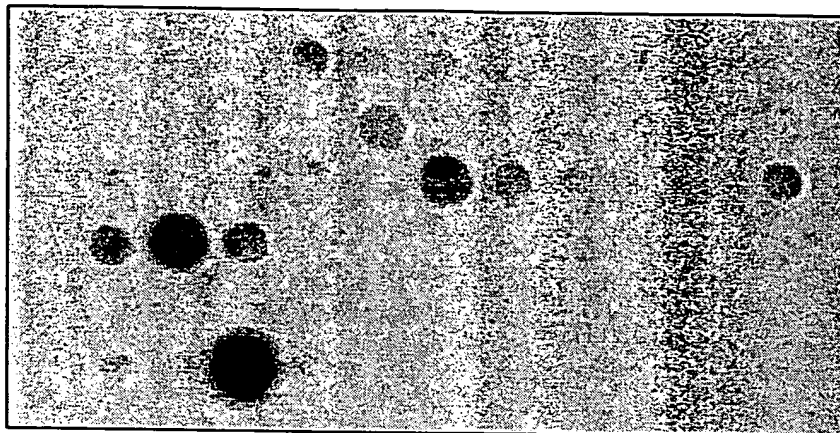
**FIGURE 50** Immunoblot of purified recombinant Ara h 3  
with serum IgE from individual patients



A

Amino Acid *Ara h 3*

1-103  
97-199  
193-295  
289-391  
385-487  
481-510



B

ISFRQQPEEN	ACQFQRLNAQ	<del>RPDNRTESEG</del>	<sup>R1</sup> <del>CYTFETWNPNN</del>	<del>OEFECAGVAL</del>	50
<del>SRLVIR</del> RNAL	RRPFYSNAPQ	EIFIQQGRGY	FGLIFPGCPR	HYEEPHTQGR	100
RSQSQRPPRR	LQGEDQSQQQ	RDSHQKVHRF	DEG <del>DLTAVPT</del>	<sup>R2</sup> <del>CVTFWLYNDH</del>	150
<del>DTDV</del> VAVSLT	DTNNNDNQLD	QFPRRFNLAG	NTEQEFLRYQ	QQSRSRRRS	200
LPYSPYSPQS	QPRQEEREFS	PRGQHSRRER	<sup>R3</sup> <del>AGQEEENECC</del>	<del>NIESCLTPEF</del>	250
<del>LEQAFQVDDR</del>	<del>QIVONIRGET</del>	<sup>R4</sup> <del>ESEEEGALVT</del>	<del>VRGGLRRLSP</del>	<del>DRKRRADDEE</del>	300
<del>EYDEDEYFYD</del>	<del>EEDRRRRGRGS</del>	<del>RCRCNGITE</del>	ICTASAKKNI	GRNRSPDIYN	350
PQAGSLKTAN	DLNLLILRWL	GLSAEYGNLY	RNALFVAHYN	TNAHSIIYRL	400
RGRAHVQVVD	SNGNRVYDEE	LQEGHVLVVP	QNFAVAGKSQ	SENFHEYVAFK	450
TDSRPSIANL	AGENSVIDNL	PEEVVANSYG	LQREQARQLK	NNNPFKFFVP	500
PSQQSPRAVA					510

Fig. 51

A.

1 2 3 4 5 6



B.

	EEEYDEDE	YDEEDRRR	GRGSR
1.	EEEYDEDE	YDEED	
2.	EYDEDE	YDEEDRR	
3.	DEDE	YDEEDRRRG	
4.	DE	YDEEDRRRGRG	
5.		YDEEDRRRGRGSR	
6.		YDEEDRRRGRGSRGR	

Fig. 52

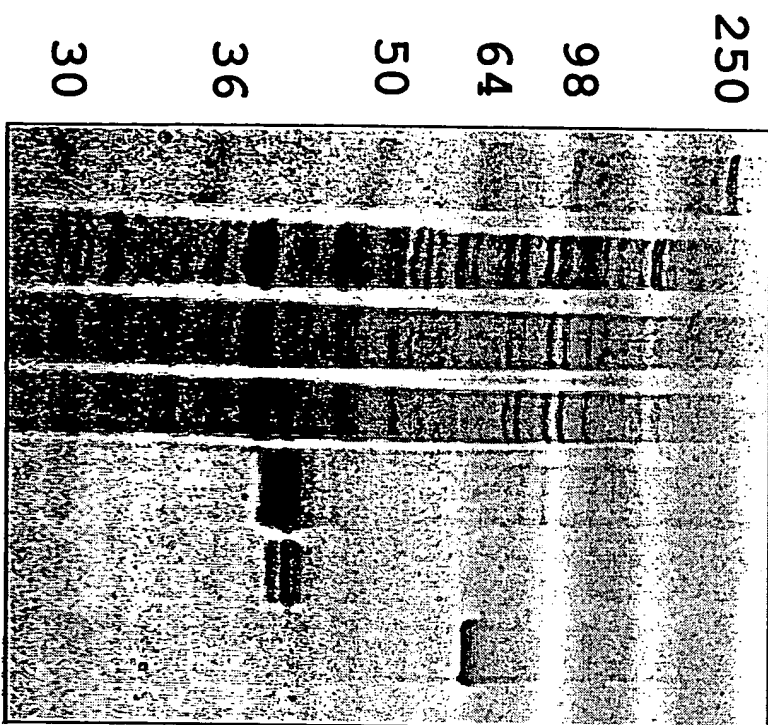
**WT**

Fig. 53

**FIGURE <sup>54</sup>** Recombinant expression and Western blot analysis of the Ara h 3 mutant

**A.**

ladder  
vector-4  
mAra h 3-U  
mAra h 3-4h  
mAra h 3-E1  
mAra h 3-PBS  
WT Ara h 3



**B.**

ladder  
vector-4  
mAra h 3-U  
mAra h 3-4h  
mAra h 3-E1  
mAra h 3-PBS  
WT Ara h 3

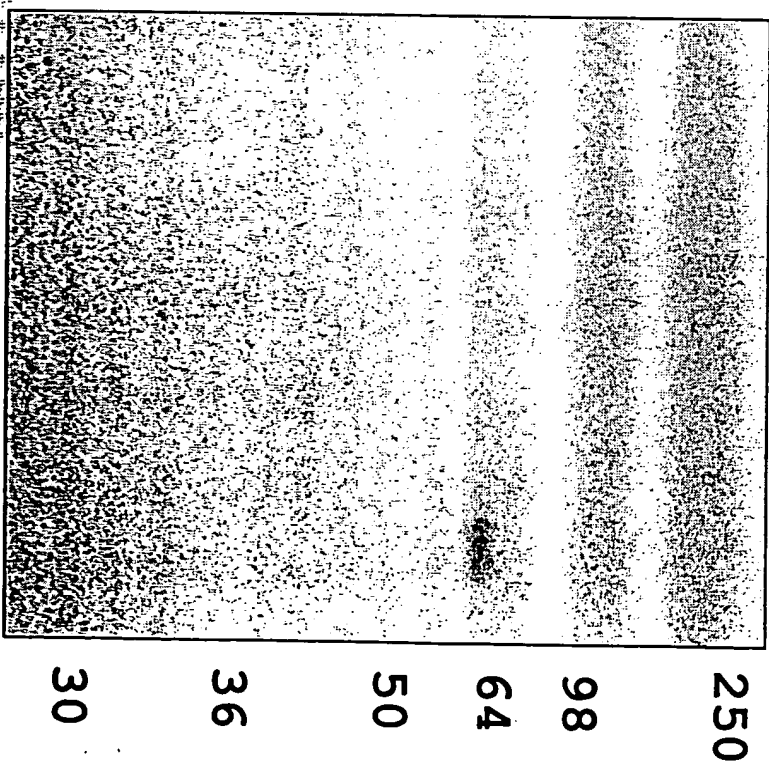


Fig. 55

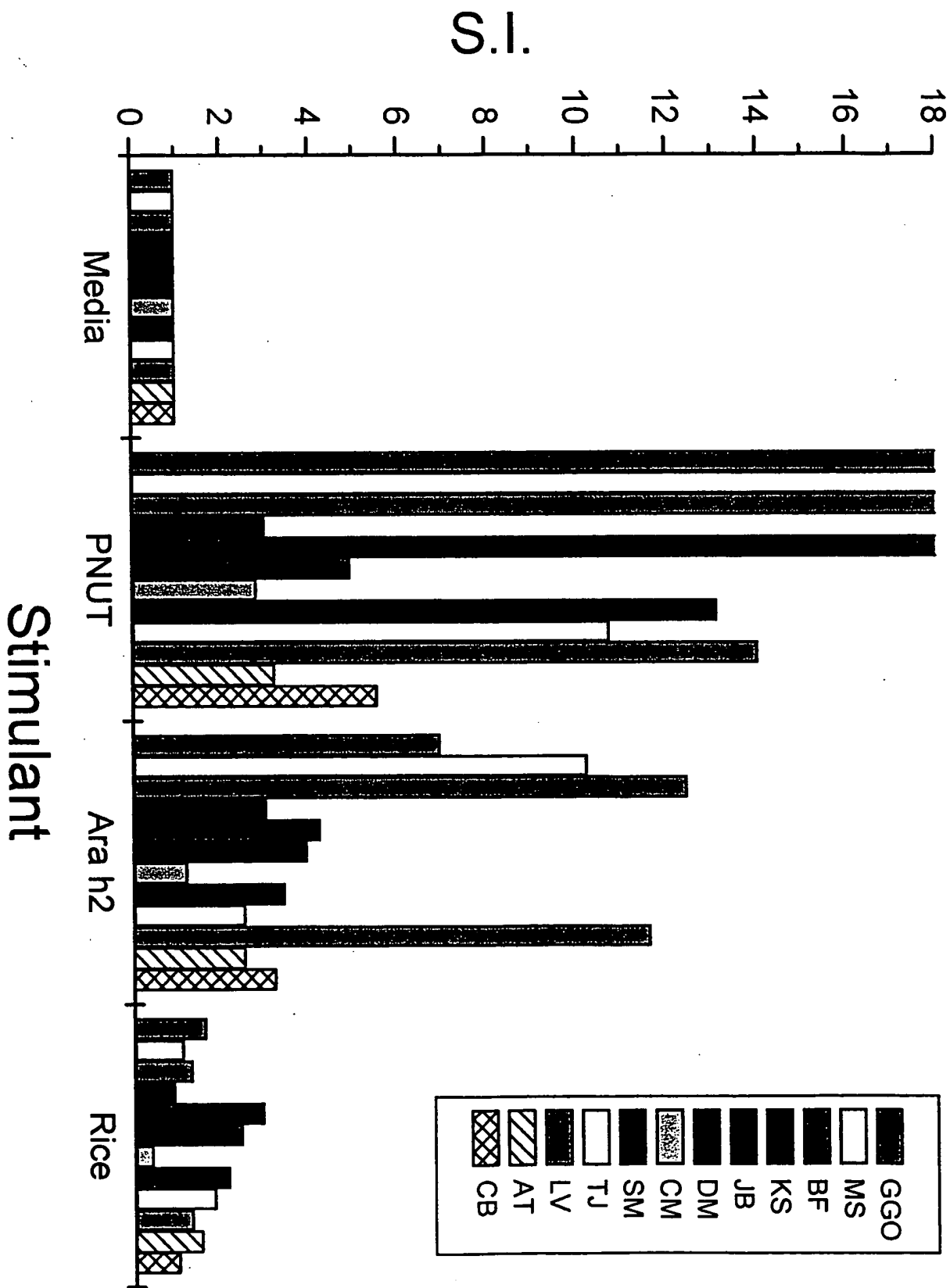
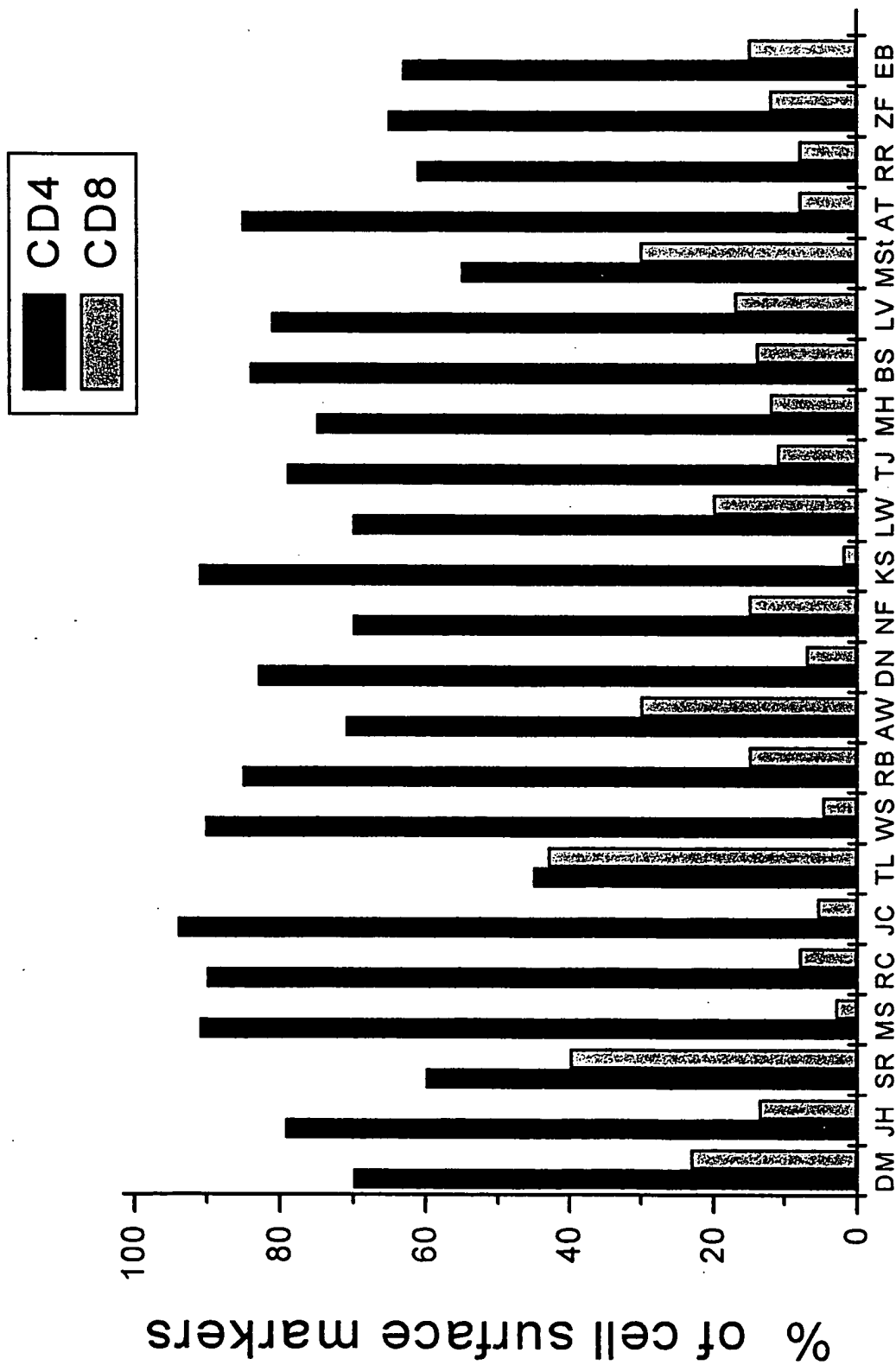


Fig. 56

68 66 64 62 60 58 56 54 52 50 48 46 44 42 40 38 36 34 32 30 28 26 24 22 20 18 16 14 12 10 8 6 4 2 0



peanut allergic individuals